IMPLEMENTATION OF PHYSIOLOGIC PRESSURE CONDITIONS IN A BLOOD VESSEL MIMIC BIOREACTOR SYSTEM

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Title: IMPLEMENTATION OF PHYSIOLOGIC PRESSURE CONDITIONS IN A BLOOD VESSEL MIMIC BIOREACTOR SYSTEM FOR THE EVALUATION OF INTRAVASCULAR DEVICES

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ABSTRACT

Implementation of Physiologic Pressure Conditions in

a Blood Vessel Mimic Bioreactor System

Kevin Mark Okarski

Tissue engineering has traditionally been pursued as a therapeutic science intended for restoring or replacing diseased or damaged biologic tissues or organs. Cal Poly's Blood Vessel Mimic Laboratory is developing a novel application of tissue engineering as a tool for the preclinical evaluation of intravascular devices. The blood vessel mimic (BVM) system has been previously used to assess the tissue response to deployed stents, but under non-physiologic conditions. Since then, efforts have been made to improve the vessel and bioreactor's ability to emulate *in vivo* conditions. The ability to tissue engineer constructs similar to their native tissue counterparts is heavily reliant upon controlling the environment and mechanical stimuli the construct is exposed to. Mimicking physiologic conditions influences cellular growth, proliferation, and differentiation. Two important mechanical stimuli are cyclic strain and wall shear stress. Previous work sought to improve these factors within the BVM bioreactor and resulted in the implementation of pulsatile perfusion and increased fluid viscosity. These previous bioreactor design modifications generated pulsatile pressures of approximately 80 mmHg and a wall shear stress of 6.4 dynes/cm^2. However, physiologic pressure waveforms were not achieved.

Studies in this thesis were carried out to implement an effective means of establishing a more physiologic pressure wave within the bioreactor that is accurate,

consistent, and easily adjustable. As a result of conducting the present studies, modifications to the bioreactor system were made that uphold the overall goals of efficacy and efficiency. The desired pressure wave was created by setting the degree of pump tubing occlusion on the 3-roller peristaltic pump head and using a water column to backpressure the bioreactor chamber. Maintaining a desired backpressure within the system necessitated the development of a new bioreactor chamber with increased extra-luminal leak pressure resistance. The opportunity was also used to further improve upon the bioreactor chamber design to allow for 360˚ rotation to reduce cell sedimentation. Modifications to the bioreactor system required quantitative evaluation to assess their impact upon local flow dynamics to the tissue construct. A system model was created and evaluated using computational modeling.

Through the work performed in this thesis, pulsatile pressure waves of approximately 120/80 mmHg were successfully established within the bioreactor. The ability to accurately model physiologic pressures will ultimately help yield tissue constructs more similar to native tissues – both healthy and pathological. The newly designed bioreactor chamber and computational model for the system will be helpful tools for implementing or evaluating future bioreactor developments or improvements. While the main objective of the thesis has been completed by creating a system capable of emulating physiologic pressure fluctuations, there still remains room for further improvements in back-pressuring and scaling the system, refining the rotational bioreactor chamber design, and building upon the complexity and accuracy of the computational model.

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Chapter 1: INTRODUCTION

<u>Cardiovascular Physiology: Blood Vessels</u>

It is impossible to engineer a tissue without an understanding of its native physiology. The cardiovascular system essentially includes the heart and blood vessels. For the purpose of this thesis, the focus is primarily on arteries. Larger blood vessels (Figure 1.1), such as arteries, are comprised of three separate layers: the tunica intima, tunica media, and tunica adventitia (33, 34). The tunica intima consists of a monolayer of endothelial cells supported by connective tissue. This monolayer lines the inner lumen of the vessel and is thus the blood contacting surface. The individual endothelial cells are sealed together by tight junctions. Tight junctions play a role in mass transport as they restrict diffusion of large molecules across the endothelium (15). Endothelial cells control vascular permeability, vasoconstriction, angiogenesis, and regulation of coagulation (15).

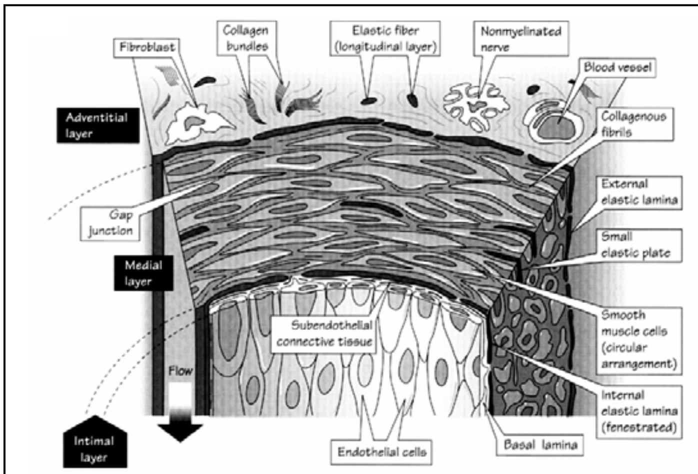

Figure 1.1 Visual illustration of the multiple layers that make up a blood vessel (34).

The tunica intima's thickness is proportional to vessel size. Larger vessels have thicker layers, but the layer still is only a monolayer of endothelial cells. The larger vessels even contain some smooth muscle cells (SMCs) within this innermost layer (34, 38). The basal lamina of the tunica intima is primarily composed of type IV collagen. The tunica media is the middle layer of the vessel. It is separated from the tunica intima by the internal elastic lamina, which is a fenestrated sheath composed primarily of elastin (15). The tunica media is made up of SMCs supported by extracellular matrix (ECM) made of mostly type III collagen, elastin, and proteoglycans (4, 20, 38, 39). The SMCs are shaped liked irregular cylinders with tapered ends and are typically between 15-100 μm in length. The SMCs are oriented in a low-pitch spiral which allows the vessels to constrict when the cells contract. While endothelial cells have tight junctions, adjacent SMCs form gap junctions. Gap junctions are characterized as areas of close cellular contact where connexons span the adjacent cell membranes (34). These channels allow ions to flow between cells and play an important role in intercellular communication (34). The cells thus form a syncitium where depolarization can spread from one cell throughout the entire network of SMCs. The third and outermost layer, the tunica adventitia, is separated from the tunica media by an external elastic lamina. The tunica adventitia contains type I and VIII collagen which supports fibroblasts and nerves (4, 10, 20, 38, 39). The outer layer in larger arteries also contains the vasa vasorum, which supplies the vessel with oxygen and nutrients.

The function of the cardiovascular system is more complex than just an interconnected network of tubular conduits transporting fluids. The fluid transport is not steady; blood pulses with the synchronous beats of the heart and pressure waves are generated. While pulsatility and fluctuating pressure can cause blood vessel movement, vasomotion (dilation or constriction) can occur independently of the heart rhythm or internal pressure (36). When attempting to recreate blood vessels, the complexity of the system is modeled as accurately as possible using a bioreactor. The bioreactor typically contains a scaffold upon which the tubular vessel is cultivated. With some tissue engineered blood vessels, the role of the tunica media and adventitia are replaced by that scaffold (17, 22, 41, 42, 51). The scaffold provides the necessary physical support and topographical environment that endothelial and/or smooth muscle cells require to attach, grow, and to proliferate. However, just like the native physiology, tissue engineering blood vessels is more complex than just a scaffold, and successful tissue cultivation heavily relies upon environmental conditions and the ability to control them within a bioreactor system.

Tissue Engineering: Blood Vessels

Tissue engineering is a relatively new and growing field of research. Currently, promising progress is being made in cultivating blood vessels, cartilage, skin and many other types of tissue (2, 24, 44). The technology of particular interest to this thesis involves the systems that are used to generate blood vessels. Most research facilities that are tissue engineering blood vessels use perfusion bioreactors. A perfusion bioreactor is generally made up of a pump, a closed circuit loop of silicone tubing, a bioreactor chamber, and a reservoir. The bioreactor chamber and reservoir are attached to the system in-line with the silicone tubing. The bioreactor chamber contains a scaffold seeded with cells for cultivation. The reservoir contains excess media which allows the bioreactor to continuously run for extended periods of time. The pump acts as the driving force of the perfusion system by circulating the media through the closed loop.

There are many different types of perfusion bioreactors (Figure 1.2) used for tissue engineering blood vessels, with variations possible by altering the pumps, and/or the chambers. For the pump, many different styles can be incorporated into the bioreactor depending on the desired fluid dynamics within the system.

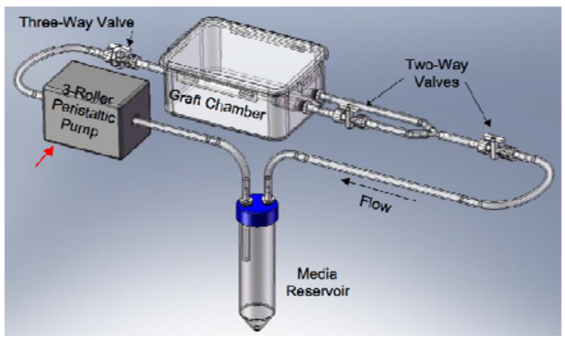

Figure 1.2 An example of a Cal Poly bioreactor for blood vessel tissue engineering.

Researchers use many different styles of bioreactor chambers as well. For example, bioreactor chambers can be designed to accommodate different vessel sizes or they can be designed with the intention of scalability. Scalable chambers (Figure 1.3) can contain multiple tissue engineering scaffolds at one time or simply conserve space (8, 22). Even the reservoirs have multiple designs that can be potentially used within the bioreactor. Most reservoirs are just simple containers of various geometries, while others contain different styles of agitators that help ensure a homogenous mixture of media (17, 40). The material properties (stiff or flexible) of the reservoir can play an important role in dictating system conditions. A flexible wall reservoir has been used to dampen or eliminate pressure fluctuations within a perfusion bioreactor (18).

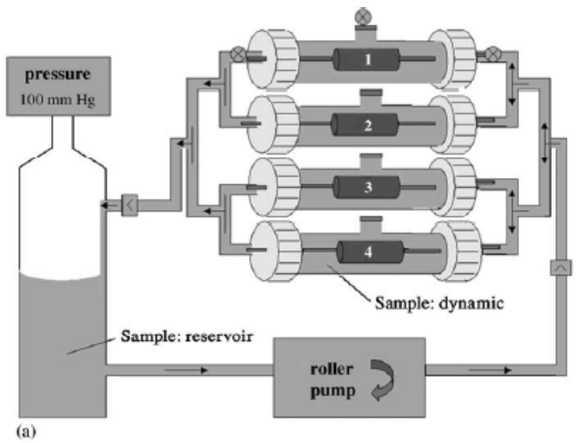

Figure 1.3 Bioreactor setup with scalable design capable of producing multiple constructs at once (14).

In order for cells to adhere or attach to the scaffolding, the scaffolding must have certain key properties, such as a similar porosity to that of native tissue (3, 27). Once cells are able to adhere to the surface of the scaffolding, the environment they are in affects their ability to proliferate and survive. Some of these conditions are easily controlled. For example, the bioreactors are maintained in incubators that regulate temperature and C02 content, which helps regulate pH. Flow rates are also easily controlled using pumps. If physiologic conditions are not emulated in the bioreactor the cells may not develop the proper phenotypic expression, alignment, or grow into a vessel with mechanical properties comparable to native vessels – if the cells survive at all (3, 5, 29). Physiologic pressures are much more difficult to recreate *in vitro*. The cyclic changes in pressure create varying hoop stresses on the walls of native vessels generating cyclic strains. These strains are believed to induce cell differentiation during vessel development and later dictate mechanical properties (43, 46, 49).

Tissue engineering, in general, consists of a basic process which includes determining a cell source, obtaining and isolating the cells, seeding or sodding cells upon a scaffold, and then culturing the cells into tissue constructs within a bioreactor. There are a wide variety of cell sources with varying potencies (ability to differentiate into other cells). In general, stem and progenitor cells constitute the cell sources for many tissue engineering applications (3, 41, 48, 54). The list of stem cells includes hemopoietic, epithelial, mesenchymal, neuronal, and embryonic. These cells can be used in their original form for tissue engineering, but they require intrinsic and extrinsic factors in order to differentiate (3). Differentiated versions of these cells are known as progenitor cells, which are also widely used in tissue engineering. Progenitor cells have already differentiated down a particular cell lineage and can divide many times.

Once a cell source and type have been selected, the cells must be accessed and isolated. Isolation typically involves enzymatic digestion or mincing and centrifugation of sample tissue taken from a specific region of the body (3). The process of cell isolation rarely yields enough cells to be able to immediately place them upon a scaffold and generate a tissue. The cells are therefore cultured and passed until enough population doublings have occurred to provide the necessary amount of cells necessary to cultivate the desired tissue. The cells are then coupled with a scaffold in one of two-ways. Cell sodding is a technique where a high number of cells are placed upon a scaffold in a blanketing fashion. Cell seeding involves fewer cells which are inserted into or onto the matrix of the scaffold (3). After cell deposition by either technique, the scaffold is then placed within a bioreactor that mimics the native conditions of the desired tissue and the tissue is cultivated for an extended period of time until, ideally, the tissue resembles the mechanical and structural properties of the native tissue.

When applying the tissue engineering process to blood vessels specifically, the correct cell type and source to use must first be determined. For example, an embryonic stem cell could serve as a cell source for the specific goal of blood vessel cultivation. An embryo is stratified into three separate layers. The layers include the ectoderm, mesoderm, and endoderm. Endothelial cells differentiate within the splachnic mesoderm that faces the endoderm (34). The process is known as vasculogenesis. The endothelial precursors assemble and form a plexus. The plexus later gives rise to embryonic and extra-embryonic vessels on the yolk sac and the placenta. The endothelial plexus expands into the somatic mesoderm through angiogenesis. The plexus will eventually grow and develop into organ-specific vasculatures under the right conditions (intrinsic and extrinsic factors). Fetal liver kinase 1 (Flk-1) endothelial progenitor cells can be derived from embryonic stem cells and differentiated into endothelial cells *in vitro* on a type IV collagen-coated dish. Vascular endothelial growth factor and VE-cadherin can also added to the system to aid differentiation (21).

The endothelial cells isolated from this particular cell source must be sodded on a scaffold. It is important to achieve a confluent and uniform cell deposition within the scaffold because it ultimately translates into proportional and uniform tissue formation. A number of research facilities utilize rotary deposition models to achieve the desired uniformity (14, 17, 22, 35, 58, 60). Failure to achieve a uniform distribution of cells may have varied results depending on the cells. Endothelial cells grow to develop a monolayer. The ECs would have to migrate and proliferate in order to redistribute uniformly across the scaffold. Smooth muscle cells, on the other hand, do not require a monolayer arrangement to proliferate and survive. As a result, presumably, the SMCs will not redistribute on their own accord as readily and this may initially result in uneven tissue growth and require longer time periods for cultivation.

With the understanding that the plexus will develop into vasculatures under the proper conditions, the next logical step would be to simulate those conditions using a bioreactor – as discussed above. This is a seemingly simple task that is much more complex than some may realize. There are a variety of environmental factors that play a significant role in cell proliferation, differentiation, and phenotypic expression. These factors can include temperature, CO2 levels, O2 levels, pH, pressure, growth factors, cell signaling, and mechanical stimulation including shear stress or cyclic strain (3, 5, 29). Having identified some of the important conditions, the next step is controlling them. This is due to the desirability of replicating physiological conditions as closely as possible in order to promote development of a tissue construct with the appropriate properties. Of the factors listed, cyclic strain and wall shear stress are particularly crucial to mechanically stimulating and engineering a vessel construct with desired physical and mechanical properties (6, 11, 18, 37, 43, 46, 49). The cyclic strain affects the SMCs and helps condition them into developing physical and mechanical properties similar to those found in native vessels. The wall shear stress affects the endothelial cells and their ability to organize, align, and mature (43, 46, 49).

<u>**Contemporary Work Engineering Blood Vessels**</u>

As the field of tissue engineering progresses, conditions necessary for cultivating replacement blood vessels are becoming better understood. Shear stress and cyclic strain have been found to be among the most important factors contributing to enhanced EC and SMC differentiation, phenotypic expression, and mechanical properties (6, 11, 18, 37, 43, 46, 49, 59). The wall shear stress is a mechanical stimulus that specifically applies to conditioning endothelial cells because they are in constant contact with the circulating flow of blood within the body. The continual exposure this stimulus implies wall shear stress is crucial factor guiding proper endothelial cell development (11, 37, 54). Shear stress has been found to promote EC differentiation with greater cell adhesion and a more confluent layer on the scaffold surface – it even promotes the proper organization and alignment of cells (37, 54, 59).

Preconditioning by cyclic strain has been found to reduce in vivo graft thrombosis and neointimal hyperplasia in decellularized arteries sodded with endothelial cells (59). Thrombosis formation can be a deadly side effect of various intravascular treatments or replacements since the clots may lead to heart attacks or strokes. Neointimal hyperplasia refers to the occlusion of the vessel. The reduced blood flow resulting from neointimal hyperplasia also has its fair share of consequences. For example, the increased resistance to blood flow results in elevated blood pressures. The cyclic strain stimulates the SMCs to differentiate and produce greater cell density, increased extracellular matrix, maturation, and organization (46, 49). It also increases the ability of endothelial cells to resist blood flow induced shear stress and fluid particulate wall adhesion (6). Since the focus of the thesis is on the implementation of physiologic pressure waves, it is imperative that previously utilized system designs and modifications are understood as well as their potential or expected benefits. Accordingly, methodologies used in the field of tissue engineering for establishing physiologic pressure waves and their effects will be explored.

Establishing Physiologic Pressure Waves

Over the past 10 years, a number of research groups have been introducing physiologic pressures in their bioreactors in order to help produce tissue constructs with more physiologic properties. In 2002, Thompson et al. incorporated a mechanical ventilator into their bioreactor. The goal of the study was to establish physiologic pressure and flow conditions. The ventilator generated both pulsatile flow and physiologic pressure waves (Figures 1.4 and 1.5) that more closely resembled the waves generated by the heart rather than a peristaltic pump (52). In essence, having achieved their goal, the researchers intended to further study the effects of these more realistic pressure waves and fluid pulsatility upon cell signaling and tissue cultivation.

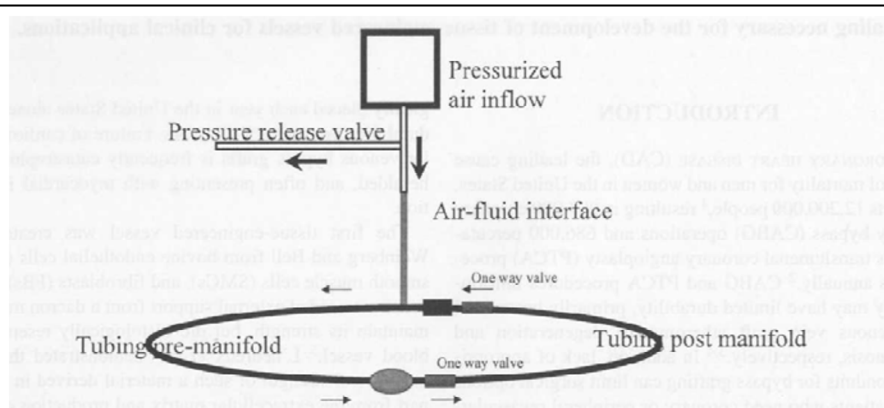

Figure 1.4 Initial concept by Thompson et al for their bioreactor design (52).

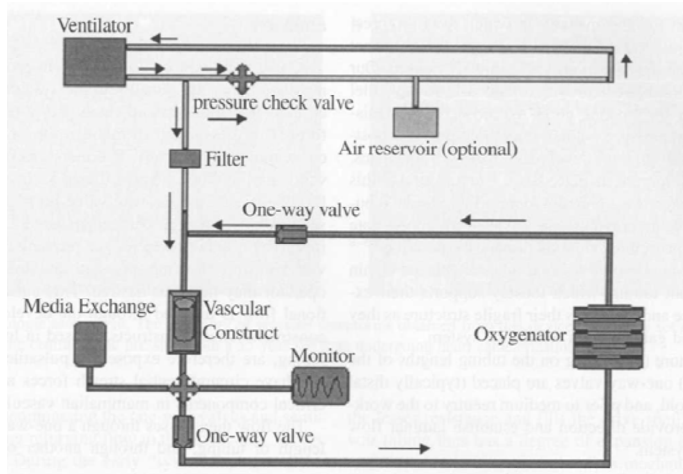

Figure 1.5 The actual bioreactor system used in Thompson et al study. The ventilator generates pulsatile flow and pressure within the system as the gasses impart their force upon the fluid column (52).

The research team ultimately found that their system modeled intravascular pressure conditions more accurately (Figure 1.6) than a roller-pump. They proposed that this system may be able to provide superior signaling mechanisms for the creation of blood vessels and heart valve. They intend on investigating the effects of compliance, pressure, pressure acceleration, flow, preseeding versus dynamic seeding, and duration of bioreactor cultivation on vascular tissue formation.

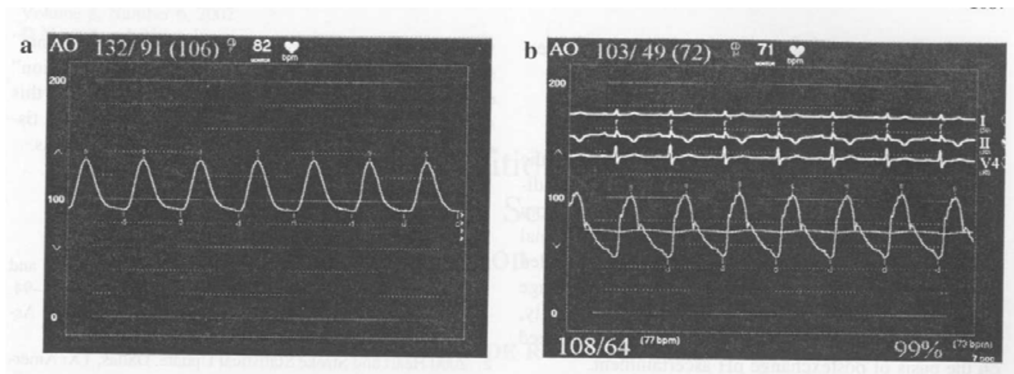

Figure 1.6 Pressure wave generated by Thompson bioreactor (a) versus an actual heart (b) (52).

Slightly more recently, Hahn et al. began focusing specifically on using physiologic pressures to help cultivate blood vessels. For their system, a Cellmax pump was placed in line with a peristaltic pump, compliant reservoir, bioreactor chamber, multiple valves, and silicone tubing. The pressure waves generated by the peristaltic pump were eliminated by incorporating a flexible reservoir. The Cellmax pumps would then impart pressure and flow pulsatility within the system. A valve was employed to restrict flow and backpressure the system. The system was able to accurately generate 120/80 mmHg pressure waveforms (18). Cellmax is commonly used instead of a peristaltic pump primarily because of the wear debris that is generated from extended use of the peristaltic pump breaking down the silicone tubing wall.

In 2005, Warnock et al. designed a sterile organ culture system for studying aortic heart valves. While heart valves are quite different from blood vessels they are both still part of the cardiovascular system as a whole. As a result, the *ex vivo* heart valves must experience similar environmental conditions of pulsatility and pressure that blood vessels are exposed to. This research team used a programmable waveform generator from Hewlett Packard to create physiologic waveforms that interface with a piston pump

(Superpump, Vivitro Systems Inc.) which imparts the wave upon the bioreactor (55). The results of this study as they relate to the heart valve are not necessarily of interest, but the physiologic wave generation is. This model is of particular interest because the pressure wave is modeled to emulate a physiologic pressure wave (Figure 1.7). The wave is not just an effect of a functioning pump that is scaled to fit the desired physiologic boundaries – the peaks and troughs span the correct range without regard to the specific waveform.

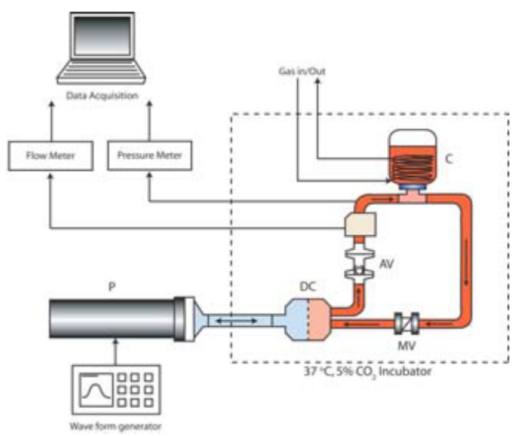

Figure 1.7 Warnock bioreactor setup for novel pulse wave generator system (55).

They found that the mechanical performance of the system allowed for independent adjustment of pressure, flow rate, and frequency. The mean pressure within the system could be adjusted to account for physiological and pathophysiological ranges (Figure 1.8 below), but the pressure amplitude is increased when pump stroke volume is increased. This issue could be avoided by using more compliant tubing for great pump stroke volumes.

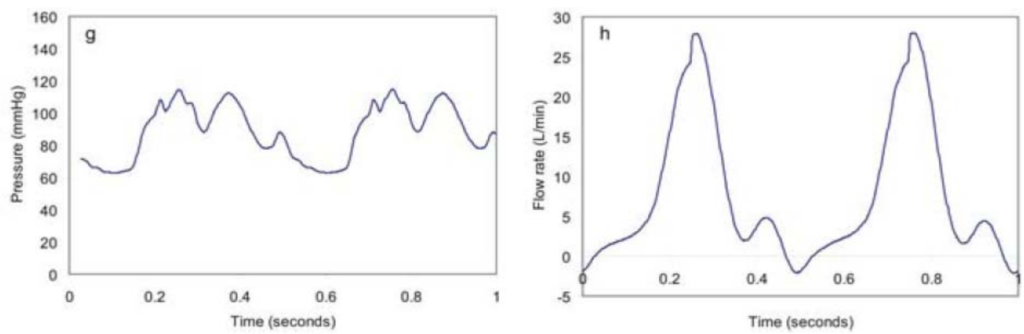

Figure 1.8 Presssure wave/flow rate measurements recorded from wave generator in study by Warnock et al (55).

Finally, in 2005, Engbers-Buijtenhuijs et al. cultured SMCs under pulsatile conditions and found that they improved mass transport of nutrients and waste to and from the cells of the vessel construct. In addition, like the other tests, tissue formation was stimulated which resulted in greater numbers of SMCs and a more uniform distribution across the scaffold. More importantly, the research team was able to establish physiologic pressures by pressurizing their reservoir to 100 mmHg and then their peristaltic pump generated a wave of 124/82 mmHg (14). The pressure was established through the application of compressed air and regulated using and electronically controlled Venturi valve. Their bioreactor is not much different than the one used in the Cal Poly BVM lab since they both utilize peristaltic roller-pumps (see Figure 1.3), and more importantly, they were able to produce physiologic pressure waves with the pump.

<u>Effects of Pulsatile Conditions</u>

The pressure within a system is related to pulsatility, and therefore pulsatile conditions will be discussed. Pulsatile conditions can be broken down into two complimentary factors – velocity and pressure. Pulsatile conditions result in fluctuating velocities. Velocity and the coefficient of friction of the endothelial cells are the primary factors dictating wall shear stress (see Equation 14.1 in subsequent section). “Q” is equal to volumetric flow rate and is determined by the product of a fluid’s average velocity and the cross-sectional area of the conduit through which it passes. Since volumetric flow is in the numerator of the equation, greater volumetric flow results in greater wall shear stress. The method by which pulsatile flow is generated within a system has an impact upon system pressure. The pressures produced within the system typically result from a pressure-specific volume relationship. For a closed system, as the volume within the system increases the pressure also increases, and as the volume decreases the pressure decreases. Decreasing the volume and increasing the pressure within the system strains the system’s walls causing them to stretch. This gives rise to the cyclic strain that blood vessels are exposed to. The amount of strain caused by the system pressure can be estimated using the equation for hoop stress (Equation 1.1).

$$T = Pr / h \qquad \text{(Eqn. 1.1)}$$

In the above equation, tension (T) is determined by dividing the product of the system pressure (P) and the radius of the vessel (r) by the thickness of vessel wall (h) (29).

The importance of these hemodynamic forces for vascular cell differentiation was recognized first in the early 1990’s. In fact, between 1990 and 1997, vascular tissue engineering studies found that cyclic strain (stretch) produced an increase in markers of differentiated smooth muscle cell phenotypes and caused changes in cellular orientation and alignment within the construct (46, 49). Further investigation into the effects of pulsatile conditions found that shear stress promoted endothelial cell adherence and increased markers of endothelial differentiation representing mature endothelial phenotypes (11, 37, 54, 59).

As tissue engineering progressed, and the understanding of the roles hemodynamic factors played in guiding tissue growth increased, studies began integrating cyclic strain, growth factors, and preconditioning to modulate SMC and EC phenotypic and cellular differentiation promotion (6, 11, 18, 37, 43, 46, 49, 59). With the variety of cell types and sources available to tissue engineer blood vessels, studies sought to understand how different flow conditions influence specific types of cells. Shear stresses were found to result in the differentiation of progenitor cells along endothelial cell lineages (41, 48, 54) and cyclic strain was found to initiate progenitor cells to differentiate along SMC lineages (4, 38). While cyclic strain and shear stress have been largely associated specifically with regard to SMCs or ECs, it is important to know that these are not exclusive relationships. There have been some effects seen in endothelial cell differentiation based on exposure to cyclic strain. Endothelial cells in cyclic strain environments have been shown to express phenotypic markers of smooth muscle cells (18).

Research has gone beyond only exploring the cause and effect relationship between mechanical stimuli and vessel tissue response and the cell signaling transduction pathways governing these effects have been characterized. The process begins with membrane signal transduction. Mechanotransduction begins with a force or stimulus imparted upon the cell. The cytoskeleton, focal adhesion sites, integrins, cellular junctions, and the extracellular matrix are then able to transmit and modulate tension within the cell. The cytoskeleton of the cell is composed of microtubules, microfilaments, and intermediate filaments that interconnect nearly all of the cellular structures. As result, structural modifications due to mechanical stimuli are capable of initiating complex signal transduction cascades that can lead to functional changes within the cell shown in Figure 1.9 (29). The stimulus activates the MAPK pathway that ends with protein synthesis. The synthesized proteins are specific to the cell type – endothelial or smooth muscle cells.

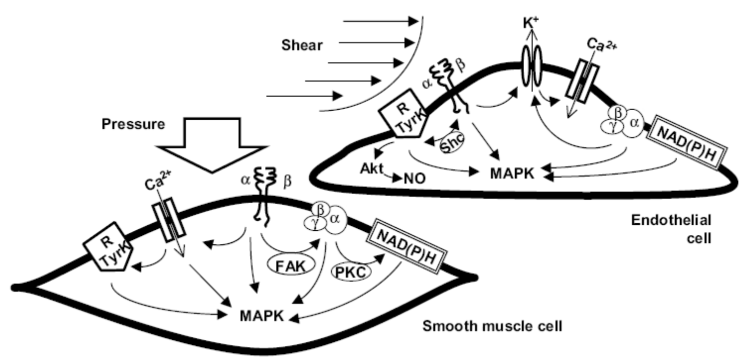

Figure 1.9 Mechanical stimulus and signal transduction (29).

In 2001, S.P. Hoerstrup et al. cultivated blood vessels under pulsatile conditions. The bioreactor included a dual-phase air pump and a “novel pulse duplicator” which they did not elaborate on, but that resulted in pressure pulses ranging from 30 mmHg to 50 mmHg in magnitude and flow rates from 125-750 ml/min - both incrementally increased over time. Endothelial cells were obtained from ovine carotid arteries and sodded upon a bioabsorbable PGA mesh scaffold. Constructs exposed to pulsatile conditions exhibited greater tissue growth than constructs cultured under static conditions. Further vessel assessment found that the pulsatile blood vessels had higher burst strength and suture retention strength (22) – ultimately greater mechanical properties (see Table 1.1 below). While the influence of pulsatility alone upon the mechanical properties of the vascular grafts cannot be discerned from this particular experiment, it is evident that flow greatly improved the mechanical properties of the vessels.

Table 1.1 Mechanical properties of tissue engineered vessels (dynamic/static) determined by Hoerstrup et al illustrating enhanced properties due to pulsatile flow.

Time in vitro (days)	Burst strength (mmHg)		Suture retention (g)		Wall thickness (mm)	
	Pulsatile flow	Control (static)	Pulsatile flow	Control (static)	Pulsatile flow	Control (static)
7	177.5 (±10)	178.8 (±4)	74.5 (±4)	67.3 (±5)	0.85 (±0.08)	0.98 (±0.08)
14	240.0 (±28)	110.0 (±18)	56.8 (±5)	42.0 (±5)	0.75 (±0.05)	0.63 (±0.13)
21	262.5 (±26)	90.0 (±8)	64.8 (±3)	25.0 (±8)	0.63 (±0.04)	0.50 (±0.05)
28	326.3 (±24)	50.0 (±5)	64.3 (±5)	12.0 (±3)	0.73 (±0.04)	0.50 (±0.1)

In 2009, Zhang et al. also cultivated vascular grafts using pulsatile conditions (60). The research team implemented a peristaltic pump to generate pulsatile perfusion. Physiologic pulsatile flow conditions resulted in enhanced tissue formation and extracellular matrix production. In addition, vessels displayed endothelial cell alignment and retained differentiated cell phenotypes. As with Hoerstrup, Zahng's experiment compared tissue engineered vascular constructs cultivated under pulsatile flow conditions to static conditions. Once again dynamic flow proves important for vessel cultivation, but the effect of pulsatility alone is not characterized. It is well established that the dynamic conditions have beneficial effects upon the tissue engineered blood vessels (60).

Summary and Goals of the Thesis

In order to further provide the motivation for this thesis, it is important to understand the future goals of the BVM lab. Laboratories and research facilities around the world are working on developing efficient bioreactor systems to grow human blood vessels *in vitro* (51, 53, 56). The primary goal of the majority of such groups, excluding Cal Poly, is to engineer tissue engineered vascular grafts for human implantation as bypass conduits (28, 35). However, the novel application of the BVM Lab's bioreactor system places this technology in a separate category of its own. It is not lab's intention to develop blood vessels for human implantation. The Cal Poly Blood Vessel Mimic Laboratory is using tissue engineered blood vessels to develop an *in vitro* assay for evaluating intravascular devices and drugs, with the goal to eventually provide results that are predictive of those obtained *in vivo*. The existing BVM bioreactor that is currently used to cultivate tissues within the lab is simplistic compared to more intricately designed systems used to make vessels for grafts (14, 22, 24). Although the Cal Poly bioreactor may not be as complex as other systems currently in operation, the system is intentionally simple to allow for scale-up, and is progressive in its intended use as a preclinical testing device.

The use of a bioreactor system to emulate *in vivo* conditions and produce predictive results is a new application of blood vessel tissue engineering. Creating such a testing model would reduce the cost and time associated with preclinical trials that rely on animal models. The BVM system could be a beneficial tool for the medical community and intravascular device companies. As this application is refined by the Cal Poly BVM lab, it can be used to gather preclinical testing data for safety and efficacy. Currently, preclinical trials include bench top testing (*in vitro*) and animal testing (*in vivo*). These trials are performed to ensure or establish both safety and efficacy of a device before clinical trials, as required by the FDA. Animal testing is the gold standard for preclinical device assessment and must be satisfactorily completed in order to move on to clinical trials. Such studies generally take 2-3 years and can cost anywhere from $10-20 million (26). Devices must undergo escalating phases of testing regulated by the FDA before they can be approved for the market. Rabbits (Figure 1.10) and pigs are typically used for animal testing involving stents (13, 32).

Figure 1.10 Rabbits are one of the animals used for assessing intravascular devices because of their vasculature, size, and regenerative properties.

During these trials, stents are deployed in the various animals and excised at different points in time over the course of the trial. The excised stents are then assessed for biocompatibility and performance. There are two main problems associated with the animal model for device testing. The first problem is that animals are not humans and they do not provide an entirely accurate example of what will occur when the device is implanted into a human patient (1, 13, 32). The other problem is the high cost associated with animal testing. The cost of purchasing animals for testing varies, but according to Charles River Laboratories, the least expensive available rabbit for purchase is $92.35. When size and sex are taken into consideration and are specifically required for a trial, the rabbits can be as much as $200.35 each (Charles River: Products and Service Guide). These prices are simply for the animals and do not include costs associated with resources, surgery, facilities, and care which are mandated by Insitutional Animal Care and Use Committees (IACUC).

The BVM system could potentially provide additional useful information more characteristic of the human response since human cells would be used to culture the blood vessels. The bioreactor system would also help reduce any unnecessary animal testing – animals are expensive and there are some ethical issues at play. This is not an indictment of animal testing, but a concern for efficiency as it relates to time and expenses and upholding the humane treatment of animals for testing.

Original BVM System

To provide the context for which this thesis is based and the general technological progression of system design, it is necessary to understand the history of the Cal Poly BVM bioreactor. The system is intended for preclinical testing and had its beginnings at the University of Arizona. Dr. Kristen Cardinal began working with the original bioreactor system as a part of her graduate studies and dissertation. Since that time, a number of years have been dedicated to the development of the technology. When Dr.

Cardinal began teaching at Cal Poly, she was given permission to bring the tissue engineered blood vessel mimic project and research with her. In 2007, the BVM Lab consisted primarily of Dr. Cardinal and four students and has grown to include 12 students today.

The original bioreactor system design was made up of a peristaltic pump with an 8 roller pump head, silicone tubing, luer lock fittings, an ePTFE scaffold, an acrylic bioreactor chamber (Figure 1.11), and a 50 ml flask style reservoir. This system ran using a continuous flow style pump and operated at the pressure induced by the pump. Pressure waves generated by the pump tubing occlusion were so small compared to physiologic pressures that they were largely disregarded and flow was considered “steady”. The acrylic bioreactor chamber used in this setup was successful for much of the previous research, but had several features that could be improved upon. The chambers were difficult to manufacture, not scalable, and challenging to assemble using sterile techniques.

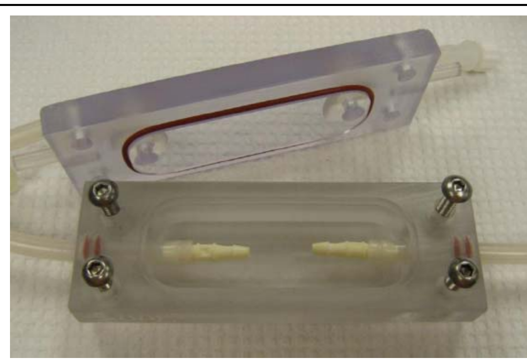

Figure 1.11 Acrylic bioreactor chamber from original bioreactor system.

Students working in the BVM Laboratory have performed a substantial amount of research and design in order to develop a bioreactor that is both easy to use and effective for engineering blood vessel mimics. While a number of students are conducting research, much of this work depends on the quality of the bioreactor providing them data and results to analyze. Sara Leifer and Marc Dawson dedicated their time to improving the bioreactor.

Sara Leifer's goal was to design a more practical bioreactor based on the system previously used by Dr. Kristen Cardinal. The older system was tried and true through the research conducted by the tissue engineering lab at the University of Arizona. A drawback to the previous system was the inability to stack the bioreactor chambers. Other drawbacks included having to fabricate the individual chambers and the small extra-luminal space. The small extra-luminal space made securing the luer lock fittings and scaffolding difficult. As a result, running multiple systems would take up an excessive amount of space. Sara wanted to create a system that could accommodate a wider range of stent lengths and diameters. It was also important to make it easy to insert scaffolds and seal the system. Sara also wanted to build a strong foundation for future research and experimentation with the bioreactor by being able to test various vessel geometries such as bends and bifurcations.

Sara evaluated various designs using the criteria as follows: user friendly design, easy scaffold insertion and removal, easy to seal/leak-proof bioreactor chamber, adjustable scaffold length and diameter, ability to be sterilized with ethylene oxide gas, optically clear bioreactor chamber, easy to scale-up and space effective, low cost, easy to manufacture, use of readily available materials, and use of biocompatible materials.

Using these criteria, Sara narrowed down her design selection to the Lock N Lock bioreactor chamber connected in series with a reservoir and peristaltic pump. This BVM system is now used to conduct testing and is the basis for new research testing different scaffold geometries, assessing electrospun scaffolds, and simulating the effects of different pathologies. The Leifer system is illustrated below in Figure 1.12. The Lock N Lock bioreactor chamber is a rectangular plastic container for perishable foods. Sara also researched new panel mount luer lock fittings to allow scaffold suspension and chamber tubing connections to be easily made. The entire top of the container is easily removed and gives the user enough space to attach the scaffolding without much difficulty. The design was also beneficial in that it allowed bioreactor chambers to be stacked upon one another. The conservation of space and inexpensive materials made this design scalable and feasible.

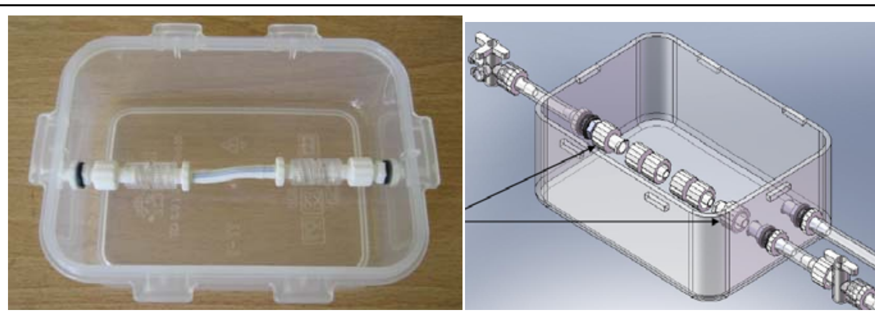

Figure 1.12 Sara Leifer's bioreactor chamber design using Lock N Lock container.

Following Sara's design and implementation of the new chamber, Marc Dawson made further modifications to the system as a part of his master's thesis. Now that the system had been made scalable and user friendly, the next avenue to pursue was emulating physiologic flow conditions. Marc worked on a variety of parameters including viscosity and pulsatility. Marc changed the peristaltic pump head from an 8 roller configuration to a 3 roller configuration. Having only three rollers made the fluid flow within the system pulse rather than continuously flow. By increasing media viscosity, Marc was able to also develop physiologic wall shear stresses within the scaffolding of the bioreactor chamber. Some additional effort was dedicated to establishing a pressure wave within the system that emulated human systolic and diastolic pressures. Progress was made in establishing a basal backpressure, but it was insufficient in physiologic terms—lacking both consistency and sustainability.

Following both Sara's and Marc's bioreactor developments, further modifications to the bioreactor system were desirable. Testing and previous experience found that the Lock N Lock bioreactor chambers suffered from leaks. Some of the leaks could be easily repaired, but the fundamental design of the chamber was not conducive to or durable enough to support physiologic pressure conditions. Consequently, it was necessary to redesign the bioreactor chamber so that it was more durable, could easily withstand a wide range of pressures, accommodate various vessel geometries, and would be compatible with future modifications and improvements upon the system. In addition to this, it was desirable to develop a method of back-pressuring the system that could be easily adjusted, accurate, and consistent for extended periods of time.

Overall, the bioreactor evolution started with Dr. Cardinal's arrival at Cal Poly and has continued ever since; with the current thesis work being the most recent addition. During this time, other members of the BVM lab have also performed numerous cell sodding, attachment, and proliferation tests to assess tissue cultivation. A well rounded understanding of the previously performed work and the future goals of the BVM lab are important to keep in mind as further developments are designed into the system.

Goals

As discussed throughout this introduction, the bioreactor plays an integral role in cultivating blood vessel constructs under appropriate flow conditions. Theoretically, a bioreactor that closely simulates physiological conditions will produce blood vessels with mechanical properties more similar to native vessels. For research in the Cal Poly Blood Vessel Mimic lab, there are two areas of opportunity that should be resolved in order to provide a sound foundation for further progress: current environmental pressure conditions, and cell sedimentation. These areas led to the development of 4 specific aims for this thesis:

1) Design a new bioreactor chamber that can withstand backpressures
2) Establish a realistic base-line pressure to mimic systolic/diastolic conditions.
3) Characterize flow conditions through computational modeling
4) Propose a methodology to prevent cell sedimentation

As discussed in the introduction, published research suggests that pressure conditions are essential to developing vessels with the necessary properties. Previous studies conducted by Marc Dawson reaffirm this assertion. The current system can accurately achieve peak systolic pressures but then drops to zero. The other issue that should be resolved is cell sedimentation. Cultured vessels could potentially exhibit uneven cell distribution with a greater cell density toward the bottom of the scaffold. An even distribution along the entire circumferential surface area is desirable.

Overall, the specified aims were addressed using qualitative analysis, quantitative experimentation, equipment provided by Cal Poly's machine shop, and by conceptual design. The original bioreactor system was characterized based on applied projected environmental conditions. Results from the initial testing were then used to illustrate potential bioreactor weaknesses and were taken into consideration when designing a new bioreactor chamber. The bioreactor chamber was produced using Cal Poly's machine shop. The new chamber was incorporated into the bioreactor and back-pressuring techniques were then evaluated qualitatively for efficacy. Potential solutions were narrowed down to the most promising option and quantitative testing was performed. Computational modeling was then used to estimate the fluid dynamics of the custom bioreactor. Lastly, a conceptual design to eliminate cell sedimentation during the seeding process was developed to provide uniform cell distributions. This work will be described in the following chapters.

Chapter 2: TESTING THE LOCK N LOCK BIOREACTOR CHAMBER

Introduction

In order to meet the goal of establishing physiologic pressure, the capabilities and characteristics of the current Lock N Lock bioreactor had to be better understood. Marc Dawson evaluated base-line pressure within the chamber as a function of flow restriction. Flow restriction on the discharge side of the positive displacement peristaltic pump increases pressure as fluid is continuously circulated through the reservoir. At a given flow rate, this flow restriction will cause the fluid to stress the walls of the system between the pump and the point of restriction. Creating the flow restriction by occluding the tubing results in increased pressure, but it is difficult to regulate since the tools used to clamp down on the tubing do not offer fine adjustment (12). The internal pressure of the system can shoot from a negligible base-line to 500 mmHg with a fraction of a turn of the adjustment on the clamping device.

Two concerns are raised by this degree of sensitivity. The first is how the system structurally responds to elevated pressure and the second is the capacity to regulate pressure. The pressure must adhere to a desired range at acceptable levels of accuracy and repeatability. Clearly, the system is constrained by reliance on variable flow restriction to generate target pressures—the concept is sound, but available hardware is too imprecise. The questions surrounding the concern of the system's response to high pressures can be readily answered by performing a leak pressure test. Therefore, the initial goals of this project – and the work that will be presented in this chapter – involved testing the existing Lock N Lock bioreactor chamber's ability to withstand physiologic pressures.

Materials and Methods

A Lock N Lock bioreactor chamber was used to conduct the chamber leak pressure test. The chamber itself is compromised of essentially two parts – the lid and the semi-rigid container. The container is made of translucent, polypropylene plastic and has locking nodes for the lid. The lid has a built-in gasket and four flexible locking arms that interlock with the nodes of the container. When the pieces are locked together, the edge of the container is pressed into the lid's gasket – sealing the two parts together. For these tests, the bioreactor chamber was assembled with the necessary luer lock fittings and the ePTFE scaffolding normally used during vessel cultivation and data was recorded using LabChart 7 data acquisition software. The data acquisition equipment included both hardware and software. The hardware included the pressure transducer, data acquisition unit, and the bridge pod amplifier (Figure 2.1). The hardware was assembled and attached to the software (LabChart 7) that was run using a Dell laptop.

Figure 2.1 Data acquisition hardware: pressure transducer (A), data acquisition unit (B), and bridge pod amplifier (C).

The next step was calibrating the pressure transducer to measure gauge pressure in mmHg. A two point calibration was performed to accomplish the task. The ADI pressure transducer for the system was calibrated with a mercury monometer. The pressure transducer was connected in line with the monometer and pressurized from 0 to 200 mmHg while the LabChart data acquisition software recorded the data. The two point calibration technique required selecting the minimum (0 mmHg) and maximum (200 mmHg) points of the recorded data to establish accurate measurements with the proper units. The system settings were saved and later confirmed using a pressure cuff. The pressure transducer was accordingly ready to be implanted within the system to record data.

The set up is illustrated in Figure 2.2 below. Three sections of silicone tubing (1/8 inch inner diameter, 1/4 inch outer diameter) were connected respectively to the one inlet and two outlets of the chamber. The two outlet tubes then converged to a single tube using a “Y” style barbed connector with an additional length of tubing after the intersection. A stopcock was placed at the exit end of the “Y” connection, as well as at the entrance of the inlet chamber tube. The stopcocks allowed the luminal and trans-luminal spaces to be flushed and filled. The ADI pressure transducer was placed in line attached to the outlet stop valve. A luer lock syringe full of water was then attached to the inlet tube stop valve and water was passed through the abbreviated system. Once water exited the pressure transducer on the luminal outlet tube, a luer plug was attached to the transducer, and the stop valve with the syringe attached was closed. The extra-luminal space of the bioreactor chamber was then filled with water. This was accomplished by removing the snap-on lid of the chamber and pouring water into the extra-luminal space surrounding the scaffold.

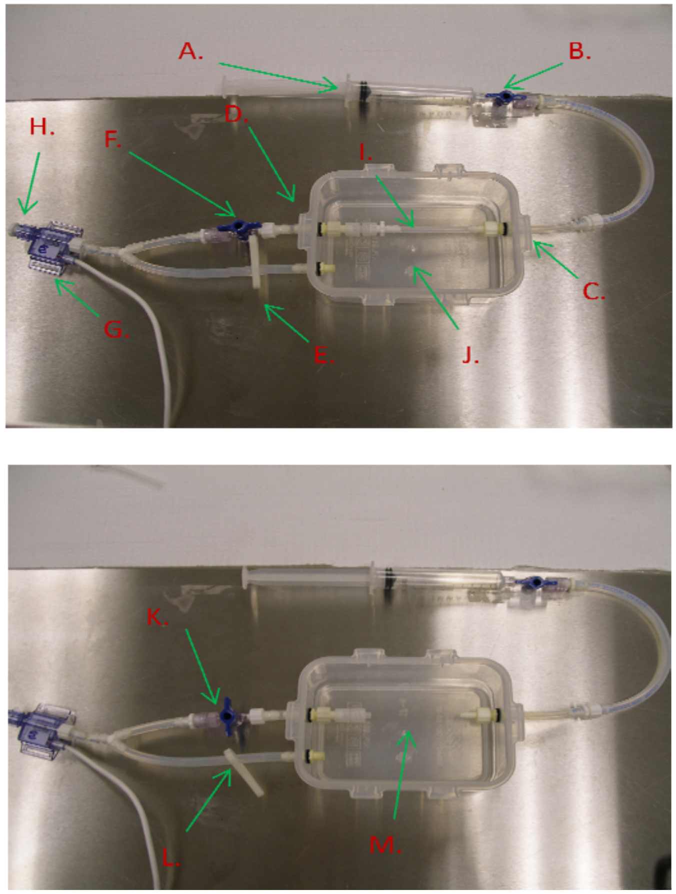

Figure 2.2 Setup for pressurized leakage test. Luer lock syringe (A) injects fluid into the system which can be sealed by the two-way valve (B) on the side of the inlet port (C). To test luminal leak pressures, the scaffold (I) is suspended within the chamber. The extra-luminal space (J) is sealed off with a tubing clamp (E). On the dual port outlet (D) side of the chamber, the two-way valve (F) is open leading to the pressure transducer (G). The pressure transducer is capped with a male luer lock cap (H). Testing the extra-luminal space (J) involves removing the scaffold (M), closing the two-way valve (K), and releasing the tubing clamp (L) on the outlet side of the chamber.

The extra-luminal outlet was closed using a hemostat ahead of the "Y" connector in order to assess luminal leak pressure. The scaffolding and clamp were removed from the bioreactor chamber to test extra-luminal leak pressures. The pressure transducer, located on the bioreactor chamber outlet, was attached to data acquisition hardware and displayed by LabChart 7 software. While LabChart recorded live data, the valve on the inlet tube was opened and the syringe, described above, was used to force fluid into the system until failure was achieved. Failure would be evidenced and defined by the onset of fluid leakage at any of the tubing connections, luer lock fittings, chamber seals, or any combination thereof.

To check the extra-luminal leak pressure, the hemostat was removed from the extra-luminal outlet and the scaffold was removed from the bioreactor chamber. Since the ePTFE scaffolding is porous, if it were included in the trans-luminal test, the generated luminal pressure would diffuse across the barrier slowly into the extra-luminal cavity of the bioreactor chamber and eventually reach equilibrium. As a result, the test would have to be performed slow enough to allow equilibrium to be reached between luminal and extra-luminal sections in order to exert the desired forces upon the rigid walls of the container. Removing both the scaffold and the hemostat allows the pressure to rapidly spread and distribute homogeneously throughout the system and stress the walls of the chamber during the test. As with the luminal test, the syringe was again used to inject fluid until the point of system failure was reached. In the extra-luminal evaluation, the definition of failure remained the same and was indicated by fluid leakage. Six leak pressure tests were conducted to characterize luminal and extra-luminal pressure limits, three tests per parameter.

Results

Three tests were performed to assess luminal leak pressure, and three tests were performed to assess extra-luminal leak pressure – six tests in total. The results of the tests are listed below in terms of pressure readings at the point of failure. Catastrophic failure for the luminal leak pressure tests was characterized by silicone tubing releasing from the luer lock barb connection. Extra-luminal leak pressure failure exhibited leaks around the panel-mount chamber wall penetrations and the seal on the bioreactor chamber lid. The common sites of failure are indicated below in Figure 2.3.

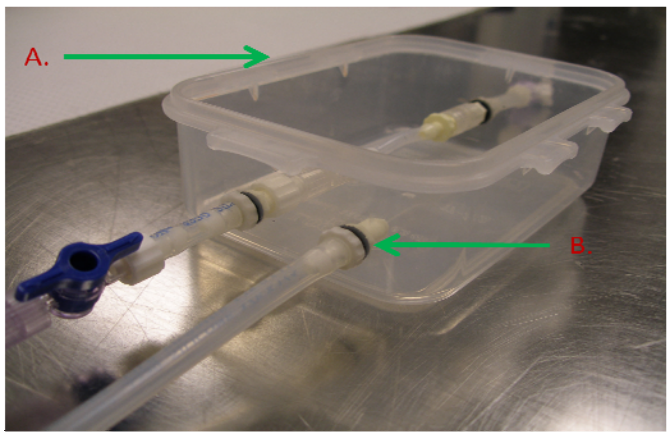

Figure 2.3 Locations of fluid loss. During the extra-luminal pressurization fluid would drip from under the lid (A) and from around the panel mounts (B).

Table 2.1 Lock N Lock leak pressure test results.

Trial	Luminal (mmHg)	Extra-luminal (mmHg)
1	1257	100
2	1204	102
3	1282	101

Discussion and Conclusion

These results are quite significant in the context of human physiologic pressure conditions. A normal human systolic and diastolic pressure ratio would be 120/80 mmHg and can range (not necessarily with good health) from 230/140 mmHg to a mean arterial pressure of 45 mmHg based on severe hypertensive and hypotensive pathologies (19, 50). The data presented in this chapter shows that the luminal leak pressure is significantly greater than this prospective range and therefore noncritical. However, the extra-luminal pressure failure threshold of approximately 101 mmHg is below the systolic 120 mmHg of an average healthy adult. This pressure limitation would hinder the ability to test pathological conditions involving high systolic pressures, or even normotensive pressures of 120mmHg, and could be a constraint in the pursuit of "optimized" pressure and flow parameters that would require exceeding 100 mmHg.

For the luminal test, failure occurred where the tubing connected to the barb of a luer lock fitting. In this case, the failure occurred at the same place each time on the extra-luminal outlet side of the "Y" connector. While the failure occurred in the same place each time, it is not necessarily indicative of a structural weakness that is located solely in that area. Any tubing and barb coupling would be at similar risk of failure. As the fluid injected into the system stretches the walls of the tubing, the barbs lose contact with the inner wall of the tube. Whichever coupling is the weakest (e.g. tubing not pressed as far onto the barb) will fail. Once failure has occurred, the resulting burst of water lubricates the subsequent couplings at that spot adding to the likelihood of repeated failures in the near future.

During the extra-luminal pressure test, leaks were seen around the inlet and outlet panel mount Buna-N O-ring gaskets. The Buna-N O-rings used to seal the wall penetrations were cracked and degraded. While any leak is bad for maintaining pressure, the leaks at each of the panel mounts were more of a nuisance in nature compared to the other source of leakage, as these leaks did not lead to significant fluid loss. This, however, could be a major issue for contamination concerns. Ultimate failure occurred at the seal of the lid to the Lock N Lock bioreactor chamber. Fluid would seemingly dribble out at the same rate it was injected in. The panel mount leaks would only become wet and produce an occasional tear.

While the luminal leak pressure is acceptable, the extra-luminal leak pressure is problematic. The tissue constructs are cultivated with the extra-luminal outlet closed and the luminal outlet open on the bioreactor chamber during normal operation. The initial sodding and preparatory stages are performed with the outlets switched (luminal closed, extra-luminal open) to force transmural flow. The pressures occurring during transmural flow are minimal and would not approach 100 mmHg and should not compromise the extra-luminal seal. During normal operation, the outlet configuration would tend to buffer the extra-luminal cavity from rapid pressure changes resulting from elevated pressures in the luminal portion of the system. However, ePTFE is porous. Eventually the bioreactor chamber will reach equilibrium with the luminal portion of the system when under constant luminal pressure. When this happens, the lid and panel mounts cannot withstand the pressures and will leak. With pulsatile flow and pressure, the trans-equilibrium pressure will be somewhere between the high and low luminal values. Given a pressure wave of 120/80 mmHg, this average would be at least 100 mmHg and very close to the failure point. Accordingly, a new bioreactor chamber should be designed to address the pressure limitation and propensity to leak of the current model. In the mean time, some temporary fixes could be used to mitigate the described failure modes, by including zip ties and silicone sealant. For example, zip ties could be applied to the bioreactor chamber to better secure the lid to the chamber with additional force. The zip ties can be applied along both the length and width of the chamber (Figure 2.4). Silicone sealant could be applied to the extra-luminal panel mount penetrations on the exterior of the chamber – covering the base of the luer lock fitting (Figure 2.5).

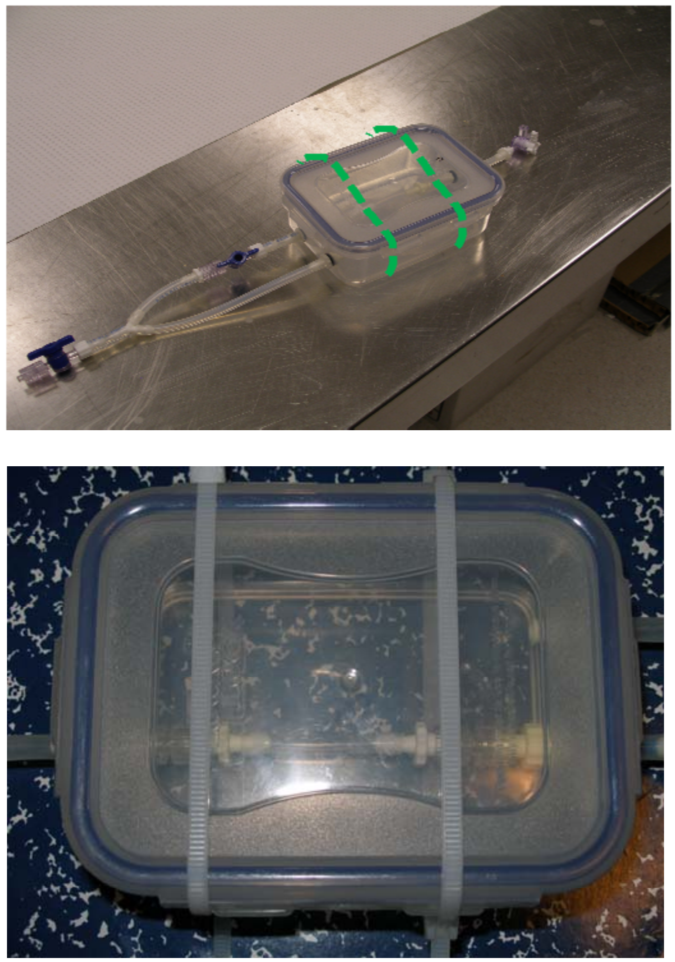

Figure 2.4 Zip-tie placement to reduce fluid loss from bioreactor chamber.

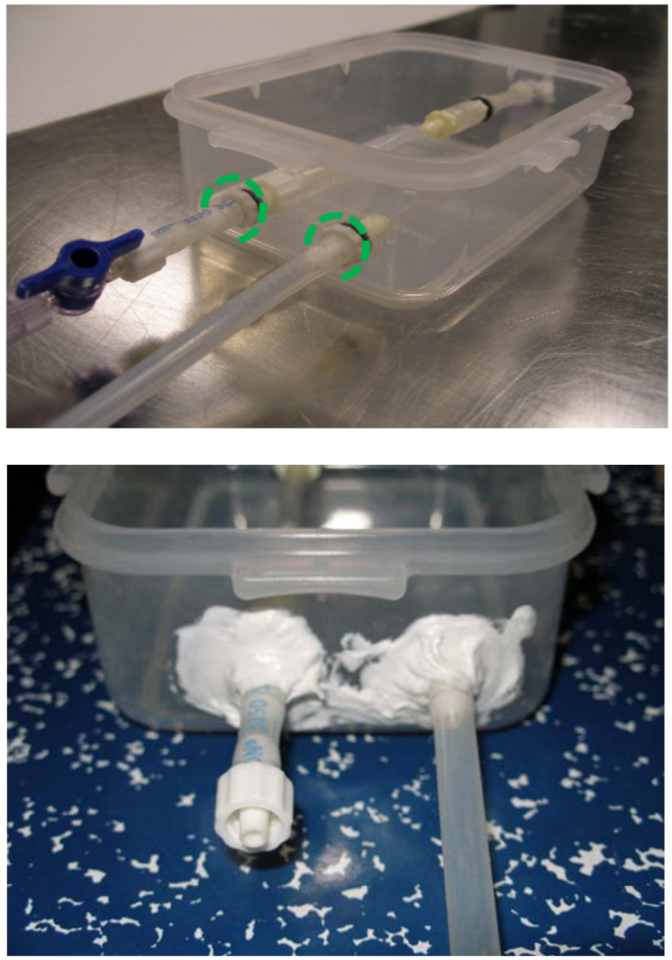

Figure 2.5 Silicone sealant placement to reduce fluid loss from bioreactor chamber.

In addition, due to the poor condition of the Buna-N O-rings, further investigation was performed. It was found that they are incompatible with ethylene oxide (EtO) gas – the method currently used to sterilize the materials (www.efunda.com). The ethylene leaches the oils from the rubber gasket. This causes the material to become brittle and crack. Every gasket inspected showed signs of degradation. The panel mounts are permanently attached to the bioreactor chamber walls with adhesive after sandwiching a rubber gasket on each side of the chamber wall. The number of times these chambers have been used is unknown. It is safe to assume that they have been sterilized more than once. Either the gasket material needs to be changed, or the panel mounts must be removable so gaskets can be periodically exchanged.

In conclusion, the work in this chapter revealed a critical problem in the Lock N Lock bioreactor chamber's inability to withstand extra-luminal pressures at or above 100 mmHg. As a result, a new bioreactor chamber must be designed.

Chapter 3: DESIGNING THE NEW BIOREACTOR CHAMBER

Introduction

The initial assessment of the Lock N Lock bioreactor chamber revealed pressure limitations that are problematic when replicating physiologic pressure wave conditions in vitro. While zip ties and silicone sealant may offer short term compensation for the problem, a new bioreactor chamber must be designed in order to reliably replicate physiologic pressures while maintaining a sterile closed system. Sara Leifer developed her bioreactor chamber to be easy to assemble and use, and to accommodate a variety of vessel geometries. In order to provide a bioreactor chamber with necessary attributes to withstand physiologic pressure conditions, some sacrifices need to be made in ease of creation and operation.

Sarah's previous work outlined a number of criteria that the bioreactor chamber must fulfill in order to be considered successful (30):

1) An overall user friendly design so that assembly is simple and straightforward
2) Easy scaffold insertion and removal without the need for bending or twisting
3) Easy to seal leak-proof chamber for quick and sterile assembly
4) Adjustable scaffold length and diameter for greater testing variability
5) Sterilizable by ethylene oxide gas
6) Optically clear chamber for easy visibility of scaffold, media and stent placement
7) Easy scale-up so that multiple chambers can be configured in the incubator
8) Low cost
9) Easy to manufacture without the need for adhesive or special tooling

10) Use of materials and components that are readily available and easy to obtain

11) Use of biocompatible materials so that cells are able to grow and function

Each and every one of these criterion is important to the design of an efficient and practical bioreactor chamber. However, criteria 1, 8, and 9 are not as important in this phase of the design process, because it is better to have a design that achieves current and potentially future goals rather than to fall short on account of simplicity and cost. In order to obtain a chamber that can withstand the necessary environmental pressure conditions, the cost of the system will undoubtedly increase. Designing a system to withstand these pressures will also likely involve a slightly more complicated manufacturing and assembly process. Within reason, these can be acceptable consequences for an improved design. While it is still immensely important that the system remain simple and easy to use, this requirement is balanced by performance expectations. The design should account for ease of use, but also achieve the important functional goal of supporting physiologic pressures.

Methods and Materials

Material Selection

As mentioned previously, there is a list of design criteria that the bioreactor chamber must fulfill. The chamber must be capable of being sterilized. There cannot be any leaks and seals must be able to withstand the entire range of physiologic pressure. It has been noted that cell sedimentation occurs during vessel cultivation. In accordance, the bioreactor chamber should be capable of being rotated 360 degrees.

Overall, the design that was created and implemented consisted of the following components: cylindrical housing, solid caps, and flexible end plates. A basic diagram of the new design is provided in Fig 3.1 below. The specific materials selected for these components will be described and justified below, followed by a detailed description of the design, manufacturing process, and assembly of the bioreactor chamber.

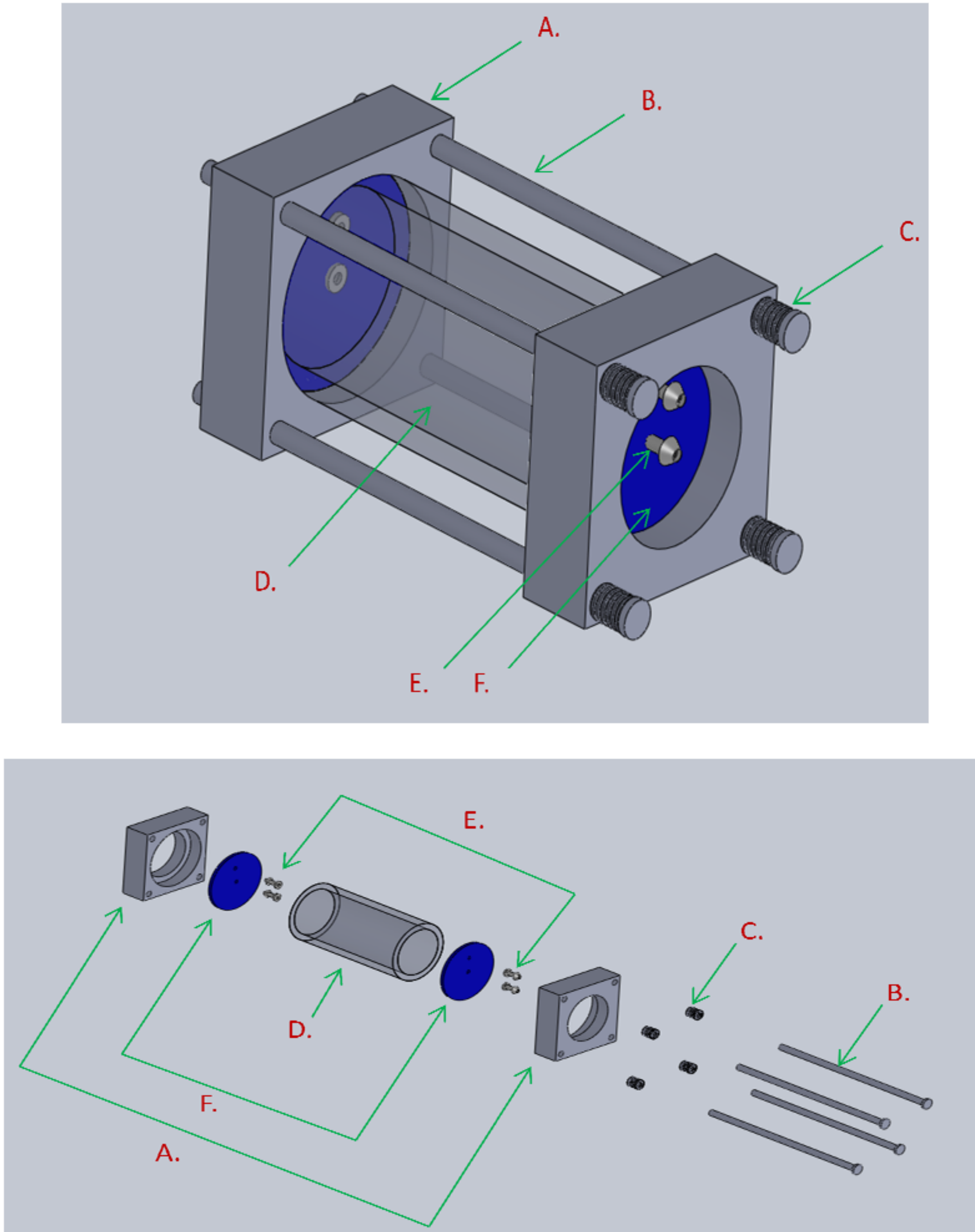

Figure 3.1 Bioreactor chamber assembly: aluminum end plates (A), bolts (B), springs (C), extruded acrylic tubing (D), luer lock panel mount fittings (E), flexible silicone end plates (F).

An extruded acrylic tube was chosen for the bioreactor chamber wall (McMaster-Carr, P/N 8486K547). The tube has a 2 inch outer diameter and a 1 3/4 inch inner diameter. Having a 1/4 inch wall thickness is important for not only ensuring that the wall can withstand the necessary pressures, but it provides structural support to allow the tightening of end caps without cracking. Acrylic is optimal because of its optical clarity, but the material is brittle. Acrylic can only withstand up to roughly 200 degrees Fahrenheit (www.mcmaster.com). Accordingly, the acrylic will have to be sterilized by EtO gas rather than the autoclave. Acrylic is a widely used material in the research community and has been shown to be biocompatible with cells (47).

6061 aluminum is an all purpose aluminum (McMaster-Carr, P/N 8975K313) that was selected for end cap fabrication of the bioreactor chamber. It is easily machined and known for its strong structural properties and relatively light weight (25). The weight of the bioreactor chamber is important for ease of use and for potential future scaling of the system. Being able to be easily machined, the metal lends itself to reproducibility. The 6061 aluminum can be sterilized using an autoclave or EtO gas. There are a wide variety of aluminum alloys that are considered biocompatible. The aluminum, in this design, will not come into contact with cells or media and therefore its biocompatibility is of less significance. The aluminum will be used to make the end caps illustrated in Figure 3.1 above.

A pharmaceutical grade polysiloxane (silicone) sheet was selected to provide stock to cut out circular end pieces for the bioreactor chamber (McMaster-Carr, P/N 5812T145). The silicone is rated to withstand a temperature range from negative 50 up to 400 degrees Fahrenheit (www.mcmaster.com). This range will accommodate the autoclave process for sterilization. The silicone end pieces were selected for their biocompatibility based on the fact that silicone tubing is used in the bioreactor. The rubber pieces also act as a gasket between the acrylic and aluminum as well as the panel mounts. This helps reduce the number of parts and potential for leaks. If future bioreactor chambers must be built, the silicone can be purchased in bulk as stamped out blanks with the desired outer diameter.

Four 7 inch bolts (Ace Hardware, 7” x 1/4”) were used to tighten down the end caps. The bolt material is not necessarily important from a biocompatibility point of view since it will not come into contact with the fluid of the system. The bolts were 1/4 inch in diameter and four nuts (Ace Hardware, 1/4”) were used to tighten down on the threads. In addition, 4 stainless steel springs (Ace Hardware, 1/2”x 1/4”) that are 1/2 inch with a 1/4 inch inner diameter were slid onto the bolts to prevent over tightening and stripping of threads. The springs had an added bonus of allowing users to evenly tighten the bolts down without inadvertently loosening nearby bolts or creating a bind.

<u>The Design</u>

The bioreactor chamber was primarily designed to withstand the entire range of physiologic pressure without leaking. The shortcomings of the Lock N Lock are largely do the inability to apply sufficient compressive force to seal the lid of the chamber, the use of luer lock panel mounts to bridge two different environments (which is not their intended purpose), and the incompatibility of o-rings with the sterilization process. These specific factors inhibit the chambers’ ability to function under the intended conditions. A secondary issue is the inability to rotate the Lock N Lock chamber. This could potentially result in cell sedimentation, and would be ideally improved by having a design that could be rotated.

The new bioreactor design addresses each of these specific issues in order to provide the bioreactor system with the necessary equipment to operate under the desired conditions, and to do so efficiently. The acrylic cylinder serves as the bioreactor chamber wall, the silicone end plates act as gaskets, and the bolt/spring/cap assembly allows sufficient compressive forces to be generated to seal the interior of the bioreactor chamber from the outside environment. This aspect of the design specifically addresses the issue of the lid seal integrity – which proved to be a critical flaw in the previous design. The issue of leaks at the inlets and outlets of the bioreactor chamber wall is addressed with the flexible silicone end plate. Even though the panel mounts are not intended to bridge different environments (and maintain their separation), selecting a silicone material with ideal mechanical properties effectively compensates for the problem. The silicone is autoclavable so there is no issue with EtO gas sterilization degrading the polymer. The silicone is also very flexible, is rated 50A on the durometer scale, and has a tensile strength of 700 psi. The silicone also acts as a gasket for the panel mounts. The flexibility and compressibility of the material allow it to stretch and accommodate the luer lock fitting while maintaining a very tight seal across its surface.

The design of the bioreactor chamber also allows for future improvements, modifications, and customizations. The flexible end plates allow multiple constructs to be assembled in a single bioreactor chamber, custom geometries and orientations of the scaffolds, and accommodation of bulkhead fittings (intended to bridge separate environments without cross contamination), which could be used to replace the panel mounts. The flexible end plates also allow for longitudinal tensile forces to be applied to the scaffold/tissue construct if ever needed or desired. The cylindrical design of the bioreactor chamber allows easy rotation of the chamber to prevent cell sedimentation. The square end caps allow manual 90 degree incremental rotations of the chamber during the sodding process. All of the benefits derived from the design are realized through the fabrication process.

Fabrication

Cutting the acrylic tube to the appropriate lengths could have been achieved a number of ways, but the method chosen was to use the horizontal band saw. The horizontal band saw uses a vice to secure the material intended for alteration in place. This is a nice feature because it allows for a straight cut. One must be careful, however, that the vice is angled appropriately. The vice can be angled to give a wide range of diagonal cuts. It is important to make sure the vice is perpendicular (90 degrees) to the saw blade after tightening the acrylic tube into place. The vice may turn slightly while securing the material in place. Although the acrylic tube is round, the vice will still hold it tightly in place. Currently, the Cal Poly Hanger has two horizontal band saws. One has a saw blade with small teeth; the other has a blade with medium sized teeth. The smaller saw teeth are good for finer cuts and more brittle materials. Accordingly, the acrylic tube was cut using the finer saw. The rate at which the saw blade cuts through the material is based upon the amount of weight placed at the free end of the band saw. There are usually two to three weights that may be positioned along the band saw to determine the rate at which the saw blade drops and cuts through the material. The weights were moved toward the saw's axis of rotation to slow down the rate at which the material would be cut.

The acrylic tube was measured using a tape ruler, and a mark was placed at 4.5 inches. The tube was measured multiple times to ensure accuracy, but dimensional tolerancing to the nearest 1/16 inch was adequate for tube length. A fine tip sharpie worked for marking the acrylic. The tube was placed into the horizontal band saw vice and secured in place. The orientation of the tube was double checked to ensure it was perpendicular to the saw. The saw blade was lowered to make sure it would cut at the marked 4.5 inches. The saw was then turned on and the tube was slowly cut through. In this case, there was a small burr that was easily filed off. The bioreactor chamber wall was complete.

Cutting the aluminum down to size for the end caps followed a similar process. The aluminum block was placed in the vice. It was beneficial to have it lying across its 1 inch height dimension because the vice had more surface contact with the block this way. The aluminum was marked off in 3 inch increments along its length using a tape measure. The measurements were taken twice to ensure accuracy. It was important not to mark off all of the three inch increments immediately, as the saw removed about an 1/8” of stock where it cuts. The aluminum block had to be removed and marked again after each cut. With the aluminum block aligned and tightened into place, the band saw was turned on. The metal was not brittle, so the speed that the saw cut the block was substantially increased. In this case the teeth on the saw were not critical to cutting the material. Larger teeth take larger bites out of the material. Since the material was 3 inches wide, only two 3 inch cuts were necessary to make two 3” x 3” x 1” blocks. The smaller blade was used to cut the material so that the cuts were clean. If many cuts were required, the band saw with larger teeth would have been used.

With the two aluminum blocks cut down to size, they were machined out using the mill. The mill provided tooling accuracy within thousandths of an inch. Unlike the dimensional tolerance of the chamber tube length, end cap bore was within ten thousandths of an inch from nominal. The aluminum blocks had to be machined to fit over each end of the acrylic tube. There were two steps to the process. First, there was a hole cut all the way through the center of each block. This hole was 2 inches in diameter. It had to match the inner diameter of the acrylic tube in order to provide a flush seal compressing the silicone end plates between the end cap and acrylic wall. Next, a 2.53 inch diameter hole was cut concentrically over the first hole. This was not a “through hole” as it only cut 1/2 inch through the block. This, in effect, created a 1/4 inch shelf for the acrylic tube to rest upon in order to provide greater surface area to seal against the silicone end plate. The outer diameter of the larger hole cut into the aluminum was slightly larger than the outer diameter of the acrylic tube to ensure the tube will slide into the end cap.

After obtaining a mill, a rotary vice was necessary to hold the aluminum in place. The rotary vice was secured to the mill’s work top using the supplied rail bolts and nuts. This type of vice was necessary to cut out large diameter holes in the aluminum. A hand-crank was used to move the vice 360 degrees. Two bisecting lines were drawn across the aluminum block at 1 1/2 inches from each edge – forming four equal quadrants. The lines should cross each other at the center of the block. Measuring out from the center, a mark was placed on each line exactly 1 inch out. These measurements are important for centering the mill later. The aluminum block was then placed in the center of the vice on top of sacrificial material. Using the x and y directional controls, the table was moved so the center of the block aligned with the center of the end mill. The end mill of choice for this application was a 1/2 inch center-cutting, 2 flute end mill.

The block was secured in place using t-nuts, bolts, nuts, end hold downs, and step blocks. The t-nut was threaded onto the bolt and then slid down the t-slot of the working surface of the vice. The end holder was placed across and edge or corner of the aluminum block to hold it in place. The step block was used to support the other end of the end holder so it could be tightened down to hold the aluminum block in place. The step block and end holder both have stepped edges that resemble teeth and were interlocked together. The same setup was used to pin down the other side of the aluminum block as well. Having centered the aluminum block on the vice and with the end mill, the work surface was moved in the x-direction until the outer edge of the end mill aligned with one of the 1inch marks. Once the block was lined up properly, the vice was rotated 180 degrees so that it lined up with the other 1 inch mark along the same line but on the other side. If the mill did not line up properly, slight adjustments were made to the position of the table in the x-direction until it did. The vice was then rotated another 180 degrees back to the original mark to check the alignment. The process was repeated until the two marks line up properly with the end mill. Next, the vice was rotated 90 degrees to align the other mark in the y-direction. The alignment was checked in reference to the end mill and adjustments were made in the y-direction until both of the marks in this plane were aligned appropriately. The end goal was to be able to spin the vice in 360 degrees and having the outer edge of the end mill pass through the center of every 1 inch mark. Once this was achieved, the end mill was aligned with one of the four marks and the digital read out was zeroed. This provided a point of reference if alignment was lost.

The milling process was straight forward, but somewhat time consuming. There was a depth gauge on the drilling portion of the mill that measures the depth of a cut. The end mill was lowered down until it almost touched the surface of the aluminum block. A small piece of paper was slid under the end mill and wiggled back and forth freely. The end mill was lowered using one hand while the other hand was sliding the paper. The end mill was lowered until the end mill barely snagged the paper and could no longer slide freely. The depth gauge was then zeroed. This was done in this fashion because the mill exerts enough force to embed the end mill into the material and give a false reading of the surface depth to be zeroed. The depth of each cut was then measured using the gauge. The RPM of the machine was set to 1700. The speed affects the finish on the cut surface slightly, but more importantly the RPM contributes to heating up the end mill. A spray bottle of coolant was necessary to keep the bit from overheating and a brush was used to clean shavings from the block and end mill. The end mill could only penetrate 50 thousandths of an inch of the aluminum block safely at a time. Larger cuts would run the risk of breaking the mill, ruining the end mill, or fouling the material. Once the set depth was reached, the end mill was locked in place and sprayed with coolant. The rotary vice was spun 360 degrees and sprayed with coolant by hand. Roughly every quarter turn the end mill had to be sprayed with the coolant to prevent over-heating. Whether the vice was spun in the left or right directions, it did not really matter. One direction was “up milling” and the other direction was “down milling”. They dictate the direction the shavings are flung and the quality of the finish. The only finish that was of particular importance was the shelf where the silicon end plate would be seated. That is dictated by the tip of the end mill and is not directionally influenced. Once a full circle had been cut, the end mill was raised and brushed off. The process was repeated going an additional 50 thousandths deeper into the material each time. This continued until the block was cut through entirely and the small aluminum slug was removed from the center.

The next step was to create the shelf in the aluminum block. From the zeroed position, the table was moved over 0.25 inches. The end result was that the end mill's edge was exactly 1.25 inches away from the center of the block. This would provide the correct 2.5 inch outer diameter required to fit the acrylic cylinder. The rotary vice was spun 360 degrees to ensure the center of the end mill aligned with the entire perimeter of the 1 inch circular edge. The same process was used to remove 50 thousands of the block with each pass. The only difference with this phase was that the machining ended when a half inch of the aluminum block was removed. This process was performed to fabricate both end caps.

Once this had been done to both end caps, it was time to drill a hole through all four corners of the two blocks. Using a straight edge, diagonal lines were penciled on the block from each corner. Then using digital calipers, the distance from the outer diameter of the hole to the nearest corner's point was measured. Each corner was measured accordingly, and a mark was placed at half the distance between the outer diameter's perimeter edge and corner point – measured from the perimeter edge. This ensured that the bolt-hole pattern would be centered relative to the acrylic cylinder and would not be thrown off by variation in side lengths of the aluminum block. These marks were used to drill the holes for the securing bolts. The measurements and marks were only performed on one of the two blocks because both blocks were to be drilled through at the same time. This helped reduce the time spent machining and ensured alignment later.

To do the drilling, the mill was fitted with the drill bit adaptor. This allowed the mill to use the various bits necessary to drill the holes in the block. Once again, sacrificial material was placed upon the vice to prevent damaging it. The two end caps were placed together – sandwiching a short acrylic tube (1 inch tall) cut from the excess acrylic material. The tube helped maintain critical alignment during the drilling process. It prevented any slippage and ensured that the bolts would align properly when the larger acrylic tube is placed between them. The end caps were not machined exactly the same dimensionally and this compensated for variations in the location of the machined holes relative to the block center-line. The blocks were secured to the rotary vice the same way as before using end hold downs and step blocks. The rotary vice was used here as well because it allowed the blocks to be moved without having to unclamp them. Unclamping would cause unwanted shifts and variation in alignment. Next, a small bit was placed in the mill drill chuck. A 1/8 inch bit was selected because it was small enough to accurately align with measured marks and sturdy enough to not break off into the material. With the smaller bit, it was important not to apply too much pressure to the bit. The bit may bend and break. The same RPM (1700) used for milling was also used for drilling the pilot holes. After the pilot holes were drilled, the small bit was swapped out for the 1/4 inch bit. The 1/4 inch bit was aligned with each pilot hole and, using the same RPM as before (1700), each hole was drilled using the larger bit. The holes were drilled using a technique called "peck-drilling" where the drill cuts through the material in quarter inch increments. The bit was brushed and sprayed with coolant in between cuts (Figure 3.2).

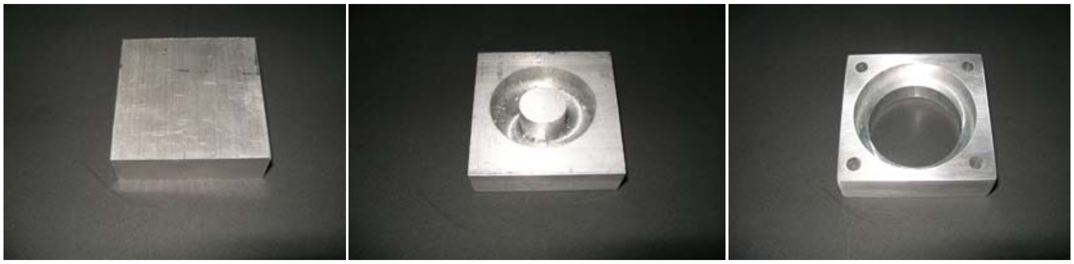

Figure 3.2 The progression of machining the aluminum end caps.

The hardware store was out of stock for the 1/2 inch steel springs that were needed. As a result, 1 inch long springs were purchased and cut down to size using a pneumatic grinder. A pair of needle nose pliers was necessary to hold the spring for safety reasons. The spring heated up considerably and the grinder itself was not gentle to flesh. Care was taken to recognize the direction in which the grinder spun because it is the same direction that the sparks are thrown. Safety glasses were worn to protect the eyes from flying metal debris.

To prepare the silicone end plates, the acrylic tube was used as a stencil to produce an outline for cutting out the disks. The silicone was cut using sharp scissors. If the design is scaled up, the silicone disks can be purchased with the desired diameters and thicknesses. A leather hole-punch was used to cut holes in the silicone end plates to accommodate the luer lock style fittings. The second to smallest punch was used to provide a snug fit for the panel mounts.

Assembly

The assembly is simple and straight forward and has been broken down into a logical series of steps. Figures 3.3, 3.4, and 3.5 illustrate completion of these steps.

End Plates

1. Take a silicone end plate and bend it in half
2. Insert the panel mount into the silicone, barb first, until the silicone reaches the threads
3. While pushing on the panel mount, screw it into place using a twisting motion
4. The stop on the fitting should be in contact with the silicone, be careful not to over-tighten and warp the plate
5. Once in place, bend the plate in half again exposing the barb end
6. Thread the nut onto the panel mount until snug
7. Repeat this process for both end plates and all the necessary holes

Dumbbell

8. Depending on the length of scaffold, add the appropriate luer lock fittings to suspend the scaffold
9. After fitting the scaffold into place, use suture to tie the scaffold down onto each barb

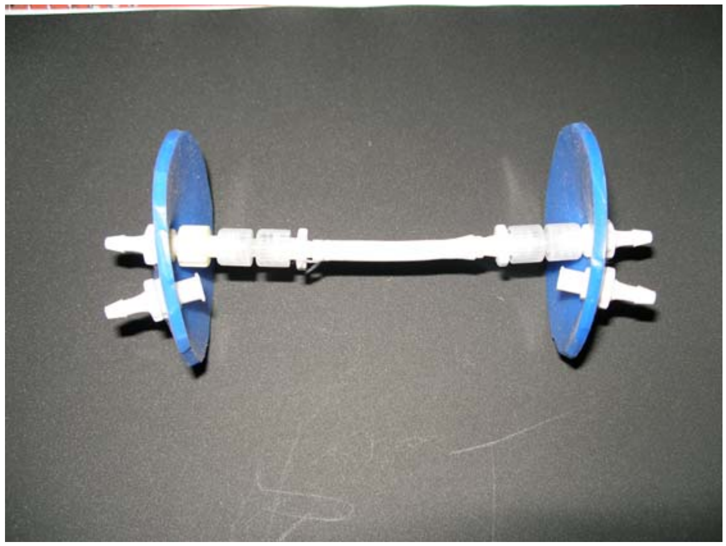

Figure 3.3 Dumbbell assembly of silicone end plates, luer lock fittings, and scaffolding.

Adding Acrylic Wall

10. Taking one end of the dumbbell assembly, bend the end plate in half toward the scaffold
11. The folded end plate is then threaded through the acrylic tube at a diagonal angle
12. The folded end plate can be grasped from the opposite end of the acrylic tube and pulled through far enough to expose the lip of the plate
13. Once the lip of the plate has cleared the end of the tube, the rest of the folded disk can be pulled out until it is flush with the tube
14. Now both end plates should align perfectly with the outer diameter of the acrylic tube

Figure 3.4 Integration of dumbbell assembly with acrylic wall.

End Caps

15. The aluminum end caps can now be positioned over the end plates and acrylic tube on both ends
16. The ½” steel springs should now be slid onto the 7” bolts
17. The bolts are then threaded through the 4 corner holes in the end caps
18. Once in place, nuts are threaded onto the bolts and tightened down

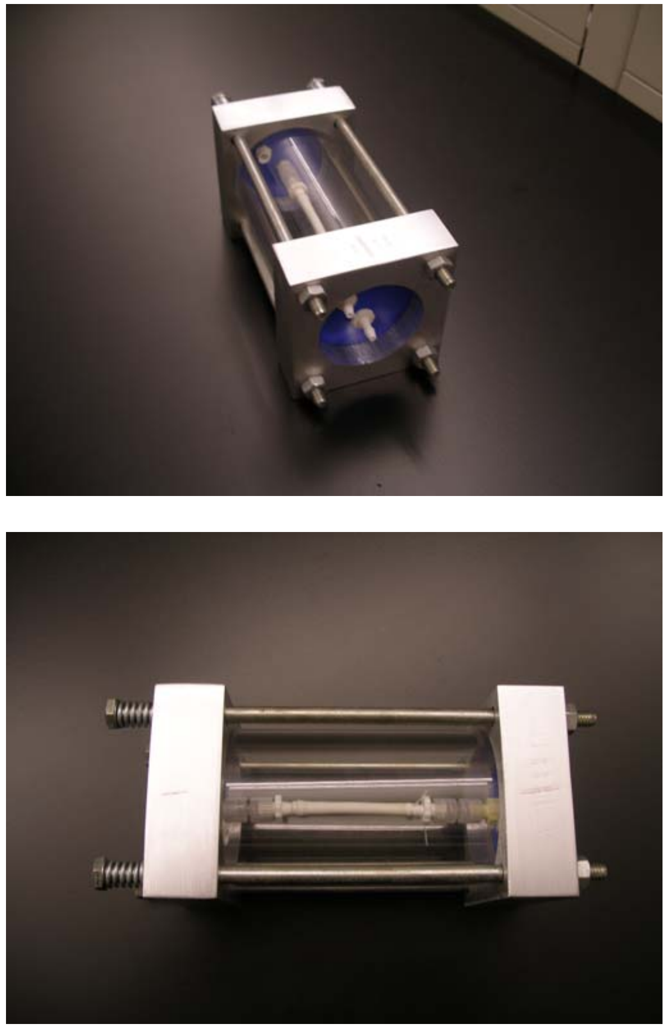

Figure 3.5 Final assembly of bioreactor chamber.

Chapter 4: CHARACTERIZING THE PROPERTIES OF THE NEW BIOREACTOR CHAMBER

Introduction

With the newly designed bioreactor chamber manufactured and assembled, tests were conducted to analyze the bioreactor chamber's capacity to accommodate a wide range of pressures. Initially, the bioreactor chamber was just evaluated at elevated pressures well above expected operating conditions. Once the chamber proved durable in these tests, a leak test was planned and performed. A leak pressure test was not conducted first with the bioreactor chamber for two reasons. The first reason was that there was only one bioreactor chamber fabricated. As a result, the bioreactor chamber was not expendable. A pressure leak test may result in a catastrophic mode of failure that would ultimately damage the chamber. It was unlikely that this would happen, but not worth the risk. The second reason was a safety concern. Once again, even though the bioreactor chamber was designed to withstand elevated pressures, there existed the extremely remote possibility of the pressurized chamber exploding. Such is the risk of having any container pressurized and the reason why warning labels are placed on products with contents under pressure. There was no reason to believe the chamber would fail catastrophically, but a conservative protocol was adopted in light of the many anticipated tests using the newly designed chamber. The silicone plates were regarded as the most likely sites of failure at high pressures. The flexibility of the silicone might compromise the seal between the acrylic tube and end cap shelves at elevated pressures near the point of failure. The panel mounts are not designed to be leak proof. They are not intended to bridge two separate environments (e.g. media and air). They also pose a threat and may leak, but the silicone itself is intended to act as a sealing gasket on the fitting. At high pressures, silicone distortion may compromise the seal between the silicone and panel mount.

Initially, the goal of this project was to use “manual pressure injection” to backpressure the system, and thus it was the focus of work in this chapter. Manual pressure injection involves straining the system by increasing the internal system fluid volume within a closed system. The increased volume would strain the walls of the closed system and generate an elevated pressure. It is similar to inflating a bicycle tire. The peristaltic pump would effectively decrease the size of the constraining system while, ideally, the internal volume remained constant. The fluctuating compressions would result in the formation of a pressure wave. Based on a finite system and volume, determining the necessary amount of fluid required to be injected into the system to achieve the desired backpressure could be easily done. The success of the technique is contingent upon the system’s ability to maintain pressure and is entirely ineffective in the presence of a leak. Therefore, assessing the bioreactor chamber’s ability to withstand elevated pressures would help draw conclusions regarding whether or not manual pressure injection would be a reasonable backpressure generating methodology. This setup did not take into consideration the bioreactor chamber’s ability to withstand pressures with more complex vessel geometries or multiple vessels. The additional panel mounts placed in the silicone end plates that would be necessary to accommodate multiple scaffolds or different vessel geometries may decrease the integrity of the bioreactor chamber seal. As a result, a separate series of tests would be needed to assess these factors and their potential influence on the bioreactor chamber’s ability to withstand pressure. Results and conclusions drawn from this study should not be extended to include the more complex configurations just mentioned.

Materials and Methods

The bioreactor chamber was assembled as described previously. One end plate contains a single panel mount (entry side) while the other has two (exit side). This simulated the setup likely to be used during tissue cultivation. 12 inches of silicone tubing was attached to the single barb of the panel mount luer lock fitting located on the entry side of the bioreactor chamber. The two barbs on the other end of the chamber were fitted with two 3 inch lengths of silicone tubing. One length of tubing was capped with a barbed female luer lock fitting coupled with a male luer lock plug. The other length of tubing was fitted with a barbed female luer lock fitting to accommodate the pressure transducer which was capped with a male luer lock plug fitting as well. The 12 inch length of silicone tubing was fitted with a two-way stop valve. A diagram of this set up is illustrated in Figure 4.1. The settings from previous calibrations could have been reused, but it was worthwhile to ensure accuracy and simply calibrate the transducer before each use. Before the transducer was hooked in line with the open system, the pressure transducer was calibrated and zeroed using a two point calibration.

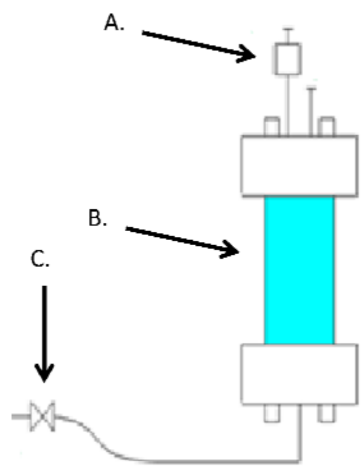

Figure 4.1 Bioreactor chamber system for pressure testing. Setup includes pressure transducer (A), water filled chamber (B), and two-way valve (C).

The luer lock plug was removed from the pressure transducer and the stop valve was opened. Using a luer lock syringe, the bioreactor chamber was filled with water. To accomplish this task, the syringe was filled with water, attached to the stop valve, and emptied into the system. The valve was closed and the syringe was removed, refilled, reattached and then emptied into the system again. The process was repeated until water shot out from the opposing end of the bioreactor chamber when the chamber was only half full. This occurred because the chamber was resting on its side. It was necessary to stand the bioreactor chamber on end to fill it completely. Once the fluid leaked out from the end of the pressure transducer (i.e. the system was completely full), it was capped off.

Before removing the syringe, the stop valve was also closed to prevent fluid leakage. The system was then ready to proceed with testing and evaluation.

The pressure range capability of the bioreactor chamber was tested first. Another syringe full of fluid was attached to the stop valve. The valve was opened and fluid was slowly injected into the system. The internal pressure of the system was monitored on the computer using LabChart 7 data acquisition software. The pressure was cycled from 0 mmHg to 1000 mmHg by pushing down the plunger on the syringe and releasing it repeatedly. The bioreactor chamber was then inspected for any signs of leakage. Next, the pressure was set at 1000 mmHg and the two-way valve was closed to maintain the pressure. The bioreactor chamber was left pressurized for 5 minutes and inspected for signs of leaks. The bioreactor chamber was then left pressurized for an hour and checked every 5 minutes for any signs of fluid loss.

The final stage of assessment involved the leak pressure evaluation of the bioreactor chamber. Testing proceeded with the leak pressure test because the bioreactor chamber had performed well as it endured the elevated pressures from the previous tests. It was determined that the leak pressure test would not jeopardize the bioreactor chamber, surrounding lab equipment, or laboratory personnel. It was also inferred that the critical leak pressure test evaluated the extra-luminal seals of the bioreactor chamber. The luminal pressure tests distributed the forces across the luminal portion of the system – stressing only the tubing and fitting connections. As a result, no scaffolding was included in the leak pressure test performed. The chamber was still assembled in the same configuration as previously mentioned. The exit side of the bioreactor chamber had two lengths of tubing – one capped and the other attached to the pressure transducer. The other end of the chamber was fitted with a length of silicone tubing with a two-way valve, a length of platinum cured silicone pump tubing attached, and another length of regular silicone tubing attached to its end. The platinum cured silicone pump tubing was clamped into the peristaltic pump. The free end of the tubing exiting the peristaltic pump was then placed into a beaker full of water. The two-way valve was opened and the pump was primed. This removed all of the air from the lines. The setup is illustrated below in Figure 4.2.

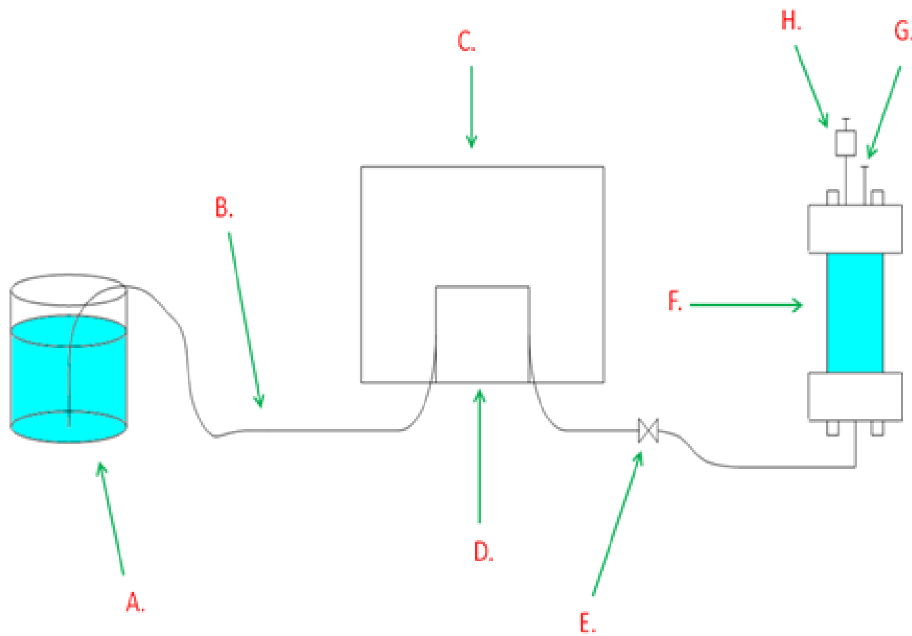

Figure 4.2 The leak pressure test setup used to fill the system with fluid and evacuate air from the lines. A beaker (A) was filled with water and the free end of the silicone tubing (B) was placed into the beaker. The silicone tubing ran to the peristaltic pump (C) and was connected to the platinum cured pump tubing which was clamped into the peristaltic pump head (D). The tubing then transitioned back into general purpose silicone tubing and was connected to a two-way valve (E). tubing connected the valve to the bioreactor chamber (F) which was stood on end and filled with water. The extra-luminal port (G) on the exit side of the bioreactor chamber was capped, and the luminal port with the pressure transducer (H) was capped after the system was entirely filled with fluid.

Once this step was completed, the RPM of the pump was increased and fluid was forced into the chamber until catastrophic failure occurred. Catastrophic failure was defined as the formation of any leak resulting in the loss of fluid from the system at any of the tubing connections, luer lock fittings, chamber seals, or any such combination. The initial plan was to use the peristaltic pump to rapidly pressurize the bioreactor chamber. It was not taken into consideration, however, that the cyclic compression of the closed system and the high RPM of the pump would generate a highly erratic pressure wave that quickly increased in magnitude until system failure. The erratic pressure measurements generated by the cyclic pump made analysis exceedingly difficult and drawing accurate conclusions practically impossible. Based on the issues that resulted from the process, the leak pressure test had to be performed by hand, just like the other trials. The manual test was performed three times in order to more accurately characterize the actual leak pressure of the bioreactor chamber.

Results

The bioreactor chamber withstood the pressure range of 0-1000 mmHg. There were no signs of leaks during cycling, but the pressure inside the chamber dropped roughly 1 mmHg in 5 minutes. After the hour long test, the system lost roughly 12 mmHg and still showed no signs of leakage. Catastrophic failure did not occur until the leak pressure tests were performed. Even then, there were no leaks from the chamber. Failure only occurred at the interface between the luer lock barb and the silicone tubing connecting to it. Table 4.1 (below) contains the recorded pressure measurements obtained during the pressure leak tests by the data acquisition equipment. The maximum pressures for each of the three tests were 1213.61 mmHg, 1215.75 mmHg, and 1196.39 mmHg - all reached within at least 11 seconds.

Table 4.1 Leak pressure test of new bioreactor chamber.

Time(s)	Test 1 (mmHg)	Test 2 (mmHg)	Test 3 (mmHg)
0	14.88	21.11	25.76
0.5	20.19	34.39	33.68
1	24.38	95.810	49.31
1.5	49.41	234.06	185.52
2	106.18	376.05	272.33
2.5	210.00	475.63	340.49
3	247.19	591.45	441.24
3.5	315.50	695.94	524.93
4	428.01	732.82	622.36
4.5	506.13	765.22	682.86
5	584.91	775.69	792.04
5.5	660.53	816.97	860.35
6	745.50	858.56	923.70
6.5	795.57	875.37	965.86
7	852.84	952.11	990.89
7.5	918.80	1018.23	1083.12
8	950.12	1066.36	1082.35
8.5	1041.07	1126.19	1139.37
9	1106.82	1144.94	1196.39
9.5	1156.18	1164.40	25.86
10	1157.15	1193.68	-1.00
10.5	1168.85	1173.40	-
11	1213.61	1215.75	-
11.5	-2.33	48.49	-

Discussion and Conclusion

Based on the results generated in this chapter, was demonstrated that the bioreactor chamber is able to operate under and withstand physiologic pressures over at least an hour. The bioreactor chamber did not show any signs of fluid loss within the range of 0-1000 mmHg during the leak pressure test or the hour-long pressurization. Thus, the newly designed bioreactor chamber is considered a success, based on the initial goal of creating a chamber that could accommodate physiologic pressure fluctuations. However, it is problematic that the pressure within the system dropped. If this system cannot hold a constant pressure, the "manual pressure injection" method will have to be changed. The dropping pressure indicated that there was a leak somewhere in the system. Wherever the leak was, it was very small, but suspicion focused on the tubing connection that failed during the leak tests. Water is incompressible. Accordingly, when small amounts of fluid are forced into closed systems, the internal pressure is greatly affected. In addition, a very small loss of fluid from a similar system would result in a large drop in pressure. There are many potential sources of leaks within the experimental setup used in this study that will have to be further explored.

In summary, the bioreactor chamber was able to withstand physiologic pressures without failing. Testing at pressures in excess of ten times human physiologic conditions were successful as well. However, the leak within the system was an issue. Therefore, the source needed to be identified and eliminated in order for pressurization of the system through manual pressure injection to be reasonable. The large number of variables within the experimental setup used in this study made determining the location or cause of the leak difficult and impractical. Although the bioreactor chamber displayed no signs of leakage, it could not be ruled out as potential source with any reasonable degree of certainty. To better understand the source of fluid loss, further testing on simplified systems with reduced numbers of variables would be necessary. This leak “diagnostic” testing will be described in the next chapter.

Chapter 5: QUANTITATIVE LEAK DIAGNOSTICS

Introduction

As stated in Chapter 4, manual pressure injection was the preferred technique to be used for back-pressuring the system. Ideally, a specified volume of fluid would be injected into the closed bioreactor system to elevate the base-line pressure to 100 mmHg – or any other desired pressure. If all bioreactor systems were built uniformly in accordance with specifications and filled with a predetermined volume of fluid, then it would be feasible to determine a specified volume to be injected to reach desired pressures accurately in all systems. The potential benefit of such a system would be that it would require minimal changes to the original Lock N Lock bioreactor setup while maintaining simplicity and scalability. The major fault with the manual pressure injection technique is that it is extremely sensitive to the presence of any leaks within the system. The study performed in the previous chapter identified the existence of a leak within a simplified experimental setup that included the newly designed bioreactor chamber, silicone tubing, and luer lock fittings. No physical evidence of fluid loss was found relating to the new bioreactor chamber within the range of 0-1000 mmHg, but the source of the leak had to be determined in order to proceed with effectively back-pressuring the system. If the bioreactor was the source of leakage, the problem could be addressed with further design modifications or improvements, and manual pressure injection would still be a reasonable method for pressurizing the system. If the leak was related to a more fundamental portion of the system, such as the tubing or fittings, manual pressure injection would require a complete overhaul of the entire system and would no longer be reasonable.

After taking a thorough, qualitative look at various simplified bioreactor models, a general understanding of the technical difficulties concerning manual pressure injection was established (See Appendix B for summaries of Qualitative assessments of various bioreactor components). The next step was to apply this understanding and perform a study to assess practical potential solutions. By determining the factors that influence pressure drop, their significance could be assessed. Then the factor, or combination of factors, that provides the greatest improvement in reducing the rate of pressure loss within the system may be determined. The intent behind this study was based on the idea that once a problem is well defined, steps can be taken to directly address it or maneuver around it.

The factors of interest for the newly designed bioreactor system included tubing permeability, and the interface between tubing and luer lock barb fittings. The silicone tubing used within the system is gas permeable to allow CO2 gas exchange within the incubator, which is maintained at 5% CO2 content. This exchange allows the media within the system to maintain a desirable level of pH. The permeability may influence system pressure by allowing trapped air bubbles to diffuse out of the system while maintaining the fluid within the system. This would cause a decrease in internal system fluid volume (gas is a fluid) stressing the constraining walls of the bioreactor. As volume inside decreases, pressure would decrease as well.

The other potential problem arises from the coupling of the silicone tubing and fittings. The silicone tubing is compliant and readily capable of deformation. The barbed tip of the fitting is slanted and focuses internal pressure in a ring around the tip – right around the circumference of the barb. Once the connection between the tubing and the barb are compromised, the tubing and fitting may separate.

In order to determine the impact these two factors have on leaks, studies in this chapter focused on applying proposed treatments on a simplified bioreactor subsystem that would impair the factors' contribution to pressure loss when compared to an uninhibited system. The simplified bioreactor subsystems are intended to reduce the number of potential variables influencing the drop in pressure. The simplified system essentially consisted of only luer lock fittings and a single length of tubing suspended between them. Based on information gained from Value Plastics technical support, the luer lock fittings are designed to be leak-proof up to 40 psi. As a result, the simplified system should only have two potential sources of leakage – the permeability of the tubing and the interface between the fitting barbs and tubing. The reservoir, peristaltic pump, and new bioreactor chamber were not included in the testing as they would introduce more variables and potentially confound results. If the tubing and connections maintained pressure, the testing would move on to include the bioreactor and more complex system designs.

Methods and Materials

In order to conduct this test, an experiment was designed with a treatment structure of two factors – each with multiple levels. Factors refer to the experimental treatments applied to the experimental unit. The experimental unit is the most basic element of an experiment to which the experimental treatments are applied. In this study, the experimental unit was the simplified bioreactor subsystem. The first factor was bioreactor subsystem modification. Factors can have multiple levels. Levels are equivalent to variations of the applied treatment. The subsystem modification factor in this study had three levels: a control, tubing spring clamps (Ace Hardware, 1/4”), and non-permeable micro tubing (HomeDepot, 1/4” x 25’). The control was necessary for comparative purposes when assessing the capacity of other bioreactor subsystem modifications to reduce pressure loss. The tubing spring clamps were used as a treatment because they showed promise as a potential solution for leaks associated with silicone tubing and luer lock barb interfaces during the primary stages of qualitative testing (Appendix B). Non-permeable tubing was used as another bioreactor subsystem modification because the silicone tubing used in the original bioreactor is permeable in order to facilitate CO_2 exchange with the environment within the incubator. It was thought that this permeability might have played a role in pressure escaping from the system.

The second factor was system size and had two levels: 12 inch (small), and 24 inch (long) lengths of tubing. Since the simplified models of the bioreactor systems were much smaller than the original bioreactors that are being used in the laboratory, it was necessary to take this difference into consideration during testing. The experimental unit was the simplified bioreactor model, and the response variable was the pressure drop over a one minute time frame.

The testing structure was a multi-factorial complete randomized block (CRB) design. Transducers were blocked on because they were potentially controllable nuisance factors that could have introduced significant variation into the test. In human studies, for example, it is generally recognized that person-to-person variation is significant and has the potential to reduce the sensitivity of a statistical test and result in a type II error. Type II errors are characterized by a failure to detect statistically significant differences between or among treatments when one, in fact, truly exists. Not knowing whether or not significant variation existed transducer-to-transducer, it was reasonable to take precautions just in case the pressure transducers did not provide uniform measurements relative to one another. As a result, transducers were blocked on in the study. Essentially, this means that each transducer used in the trial would receive all treatment combinations. This is a beneficial technique, when applied correctly, because the statistical analysis of the data can then remove the random variation attributed to each pressure transducer and compare treatment combinations without the added variability. In the end, it makes the test more sensitive to detecting significant differences between treatment combinations and their response variable measurements. All experimental data was analyzed using Minitab (statistical software) at a standard alpha of 0.05. Uncontrolled nuisance factors did exist. Water temperature and trapped gas would be examples – even though all water used came from the tap and bubbles were removed from the lines during system assembly. The potential variation the nuisance factors may have added to the test was reduced through randomization of treatment combination and transducer run orders. There were six treatment combinations because there were two factors, one with three levels and the other with two. The primary interest of the experiment was to identify which treatment or treatment combination would drop the pressure the least.

Before the main study could be conducted, a pilot study was performed in order to determine the number of samples necessary to produce results with an acceptable power. The power of a test refers to the probability that the test will reject the null hypothesis when the alternative hypothesis is true. Rejecting the null hypothesis in this case would indicate that one or more of the factors are significant at one or more of their levels, or that there is a significant interaction between factors. To conduct the pilot study, all six treatment combinations were randomly assigned to two transducers using a random number generator. The combinations were labeled one through six. The number generator would produce integers from one to six (inclusive) for one transducer until all six treatment combinations were represented one time. The run order between transducers was also randomized. The transducers were labeled one and two. The number generator produced a series of ones and twos that dictated which transducer would be used and in what order. Numbers were generated until all treatment combinations were tested for by each transducer.

While conducting the testing, the various treatment combinations were connected to AD Instruments pressure transducers, which were in turn connected to the LabChart data acquisition module and software (Figure 5.1). Each pressure transducer was independently calibrated using the two point calibration technique, and the settings were saved to separate files in order to prevent repeatedly recalibrating the transducers.

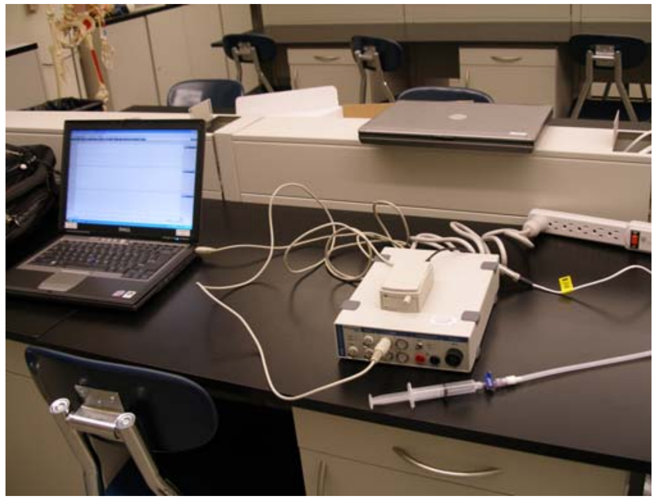

Figure 5.1 Experimental setup for pressure leak studies, including ADI data acquisition system and simplified bioreactor system (with syringe).

The signal measured by the pressure transducer was amplified using a bridge pod amplifier. The amplifier was necessary because the experiment had to accurately record measurements involving low pressures. Once the simplified bioreactor subsystem was connected to the system, it was flooded with fluid and then sealed using valves. Using a syringe full of water, additional fluid was then forced into the system to increase the pressure. Pressure readings were monitored on the LabChart software. Pressure was initially set to 110 mmHg. The system was allowed to settle until it reached 100 mmHg. The pressure drop over the following minute was then recorded. Performing the pilot study yielded 12 sample measurements – one measurement for each combination and on each transducer.

The generated data was averaged together for each treatment combination to produce mean estimates. The 12 measurements were accordingly reduced to 6 means. These means were then used to conduct a power analysis using Minitab. Based on the results, adequate sample sizes were then selected for performing the main study. The analysis was performed with the intention of establishing a power of 0.90.

Performing the main study was similar to conducting the pilot study. The only difference was in the number of treatment combinations tested and the number of transducers used. Randomization was still established using random number generated sequences. Every transducer was independently calibrated using the same method as before, and each the settings were saved to separate files for later use. Recalibrating each transducer before use would have been time consuming. The data points were collected and recorded. They were later transferred into Minitab to be analyzed. The statistical analysis of the data was performed using the General Liner Model (GLM) for a two-way ANOVA. The two-way analysis of variance (ANOVA) was useful because this experiment was assessing the affects of multiple factors and treatment levels with potential interactions. The factors and treatment combinations were then compared and conclusions were drawn.

Results

Pilot study

From the pilot study, 12 data points were gathered for the power analysis. The corresponding measurements are listed in the Table 5.1 below. The points were averaged together for each of the treatment combinations. In addition, the mean range and data standard deviation were also calculated. This information was then used to perform the power analysis in Minitab. The resulting graph is listed in Figure 5.2. Based on the experimental design, the tabulated sample size indicated the number of blocking groups required in order to obtain the desired power level of 0.90. The power analysis indicated that a sample size of six was needed. As a result, it was necessary to block on six different transducers. "Blocking" on six different transducers meant that six separate transducers would each be used to test all six treatment combinations.

Table 5.1 Pilot study pressure loss measurements for treatment combinations for each transducer.

Treatment combination	Transducer 1 (mmHg/min)	Transducer 2 (mmHg/min)
Control-12"	0.85	2.50
Non-permeable-12"	1.30	1.10
Control-24"	1.00	1.80
Non-permeable-24"	5.90	3.50
Spring clamp-12"	0.30	0.70
Spring clamp-24"	0.10	0.20

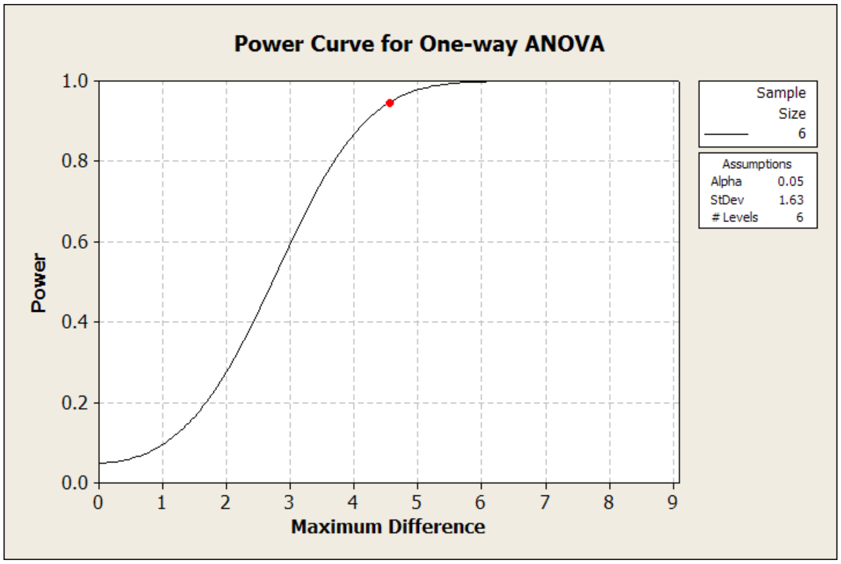

Figure 5.2 Power curve generated from pilot study, indicating that 6 transducers must be included in the main study testing each of the treatment combinations.

Main Study

For the main study, six transducers were used to record pressure drops for the treatment combinations. Since there was potential for human error, two measurements were taken for each treatment combination with each transducer. The entire data set can be found in Appendix C. Before proceeding with any analysis, the assumptions necessary for the model had to be checked. In Figure 5.3 below, normality and residual plots characterizing the data supported the necessary assumptions made to conduct the statistical analysis of the gathered data points. The linear nature of the normality plot indicated that the data was normally distributed. The ranges for the residuals on the “Versus Fits” plot were all relatively the same, and the residuals for the “Versus Order” plot were random and void of any megaphone or arcing patterns. As a result, the assumption of equal variance was satisfied. The randomization process used in performing the testing satisfies the requirement for sample independence. Accordingly, the assumptions were met and analysis could be performed.

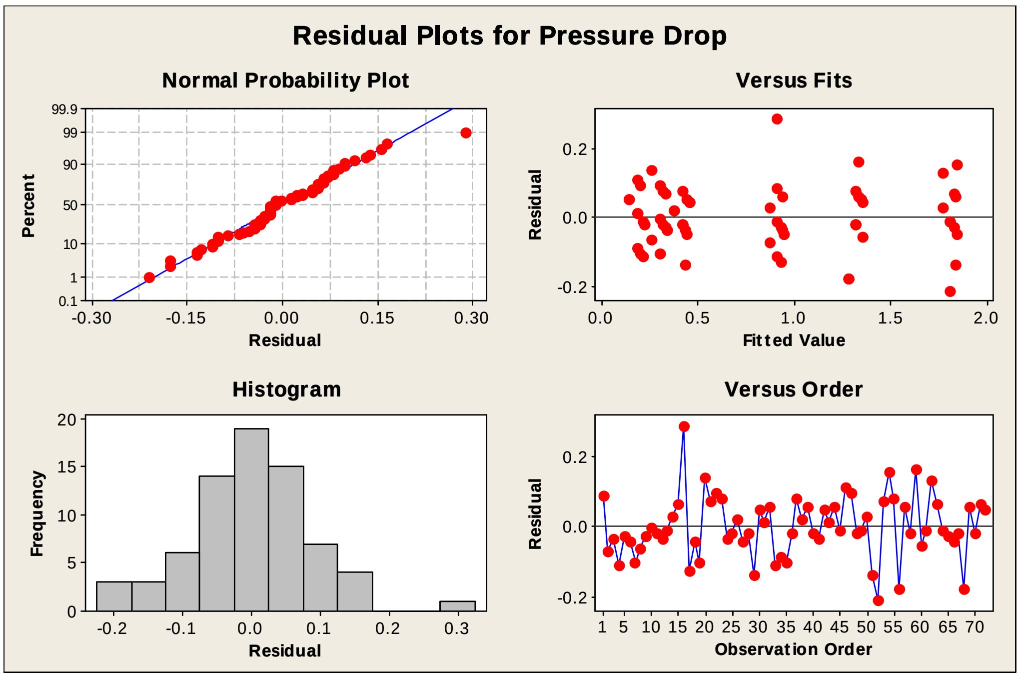

Figure 5.3 Statistical charts used for assessing data compliance with statistical assumptions.

The output from the two-way ANOVA showed that blocking on the transducer was not necessary. Since the blocking variable received a p-value greater than alpha at 0.439, the average measurements taken by each transducer were not significantly different from one another. The ANOVA also indicated that there was a significant interaction between the two factors since the combination "Size*Treatment" received a p-value smaller than 0.001. The corresponding interaction plot is illustrated in Figure 5.4.

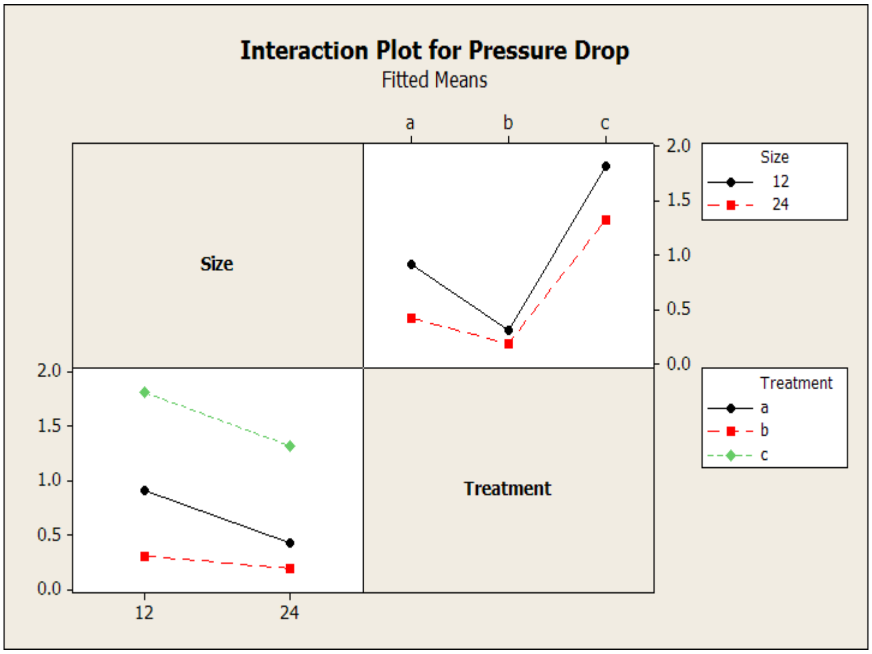

Figure 5.4 Interaction plot illustrating how system size and applied treatment affect pressure loss. Sizes 12 and 24 correlate to the 12 and 24 inch lengths of tubing used within the bioreactor subsystems. Treatments a, b, and c correspond to the control, tubing spring clamps, and non-permeable tubing respectively.

While each of the individual factors also had small p-values that were less than alpha, statistically significant interactions will always dominate the effects on the response variable. Accordingly, they did not need to be considered in any further analysis. Accordingly, while size and system modification had effects on the rate of pressure drop, they would not have an effect greater than their combination. Results indicated that the larger bioreactor subsystems lost less pressure over time compared to the smaller systems, tubing spring clamps resulted in decreased pressure loss while non-permeable tubing increased pressure loss over time compared to the control subsystem, and most importantly that the combination of large system size and the use of tubing spring clamps resulted in the lowest pressure drop of all (Figure 5.4).The treatment combinations were compared using a Tukey pairwise comparison to assess their similarities. The pairwise comparison also illustrates the results shown in the interaction plot, but in a numerical form. This data can be found in its entirety in Appendix C. All of the comparisons resulted in p-values less than alpha. This indicated that no two treatment combinations were alike. Accordingly, the lowest least square mean listed below Table 5.2 corresponds to the best treatment combination for reducing pressure loss.

Table 5.2 Least square means for factors and treatment combinations. Treatments a, b, and c correspond to the control, tubing spring clamps, and non-permeable tubing respectively.

	Mean Drop in mmHg/Min
System Modification	
a	0.67
b	0.25
c	1.57
Size	
12	1.01
24	0.64
Size*Treatment	
12 a	0.91
12 b	0.30
12 c	1.81
24 a	0.42
24 b	0.19
24 c	1.32

Discussion and Conclusion

Since there was an interaction between treatment factors, the interactions plot was the most appropriate graphical representation of the data. The two graphs illustrated the same relationship, but the size plot made the comparison easier to understand. In Figure 5.4, the black line represented the smaller sized bioreactor models, while the red was the larger. Since the black line is elevated above the red at all points, it can be seen that the smaller systems tended to leak faster. Letters “a”, “b”, and “c” refer to the control, tubing spring clamp, and non-permeable tubing subsystem modifications, respectively. For both sizes, the tubing spring clamp treatment resulted in less pressure loss than the control. It can also be seen that for both of the system sizes, the non-permeable tubing had a much greater loss in pressure than the control. The two points for the tubing spring clamp system modification were very close together. This is where the Tukey pairwise comparison became important. The comparison assessed whether or not those two points, among others, were significantly different from each other. Based on the comparison, it is clear that they are (their comparison yielded a p-value less than alpha). The difference between those two points was better represented in the interaction plot comparing treatments (Figure 5.4). The very small slope connecting the two points for the tubing spring clamp treatments showed their similarity.

The high rates of pressure loss seen for the non-permeable tube treatment were surprising. The silicone tubing typically used for the bioreactors is gas permeable. If there is any gas within the system (bubbles), the permeable tubing may allow the gas to escape and result in lost pressure. Pressure lost from this variable may be small or take an extended period of time to occur, but it was still worth assessing. Non-permeable tubing should have prevented the leakage from occurring, if it existed, and either resulted in no significant difference compared to the control or a slight drop in the rate of loss. Counter intuitively, the rate increased. This was possibly due to the material properties of the tubing and the effects on the interface between tubing and barb. When the barbs were inserted into the tubing, there was adequate tube deformation and stretch to indicate an acceptable fit. The outer diameter of the tubing was 1/4 inch, but no specifications were listed for the inner diameter. Logically, luer lock barbs are intentionally sized larger than the inner diameter of the tubing they are meant to connect to in order to ensure a snug fit. Not having a specification on the inner diameter, it was unknown whether the two pieces actually fit together properly. Once the fittings were inserted into the non-permeable tubing, however, the fittings could not be removed. On the other hand, the silicone of the original tubing interfaced well with the barb and practically stuck or adhered to the surface of the plastic. The non-permeable tubing used was vinyl and had a very smooth surface that may have compromised the seal and supported capillary action.

When comparing the square means for each of the treatment combinations, it is easy to see that the 24 inch system size and tubing spring clamp treatment combination bioreactor model reduced the rate of pressure loss the greatest. This has a number of implications. The first is that the pressure was lost in the bioreactor system at tubing and fitting connections. While clamping the tubing to the barb provided a reduction in pressure loss, it did not altogether prevent it. Either pressure was still leaking at the tubing and fitting connection, or there were other sources of pressure drop present. Since the bioreactor had been reduced to a simplified model, the other potential sources were easy to deduce. Because of the material selection and results for the non-permeable tubing, tubing permeability is still potentially a source for pressure drop. The luer lock fitting connections themselves are also potential sources of leakage. This was also noted when performing the qualitative testing described in Appendix B.

The results obtained make sense. Larger systems contain greater volumes of water. The water lost in a smaller volume system comprises a greater percentage of water compared to the larger system assuming the leak volumes in each system are equivalent. This results in a greater rate of pressure loss in the smaller systems. Also, the reduction in pressure loss achieved through tying the tubing down on the barb is reasonable when considering how the tubing reacts under pressure. As the silicone tubing is pressurized, there is a ballooning effect because of the material compliance. This ballooning can compromise the seal between the barb and tubing. The non-permeable tubing is much less compliant than the silicone. While ballooning should not be an issue for the vinyl at these pressures, the non compliant nature of the material may result in greater force concentrated at the tubing and barb interfaces. Especially when compared to a more compliant material that can expand redistribute the forces. In this case, it should not play much of a role since both systems are pressurized at relatively low levels.

Ultimately, it would not be reasonable to tie off all of the connections for the normal bioreactor. In addition, and more importantly, there are still other sources of leakage that would have to be addressed. The manual pressure injection method for back-pressuring the systems is not feasible using the either the older Lock N Lock or recently designed bioreactor systems due to their dependence on silicone tubing and luer lock fittings. As mentioned previously, the manual pressure injection technique is highly sensitive to any fluid loss within the bioreactor system. Small leaks result in large drops in pressure due to the incompressibility of liquids. Even though larger bioreactor systems and tubing spring clamps have shown to be pivotal in reducing pressure loss from the system, addressing these two issues did not entirely prevent pressure loss. Further work dedicated to improving these system fortifications would not likely yield a leak-proof system. As a result, a new method of pressurizing the systems will have to be developed to replace the previously intended method of manual pressure injection, but this is only considering the simplified systems.

The issues become even greater for identifying the many potential sources of micro leaks within the full bioreactor system. Locating and effectively resolving each of these leaks would be extremely time-consuming. Furthermore, this commitment of effort would be necessary every time the system was assembled or after any partial disassembly – too inefficient to be practical. In an ideal system, absent any leaks, forcing additional fluid into the system would effectively pressurize the system and establish a base-line pressure that would be maintained indefinitely. However, the leaks are substantial enough that fluid would have to be injected every few minutes to maintain the necessary levels in the laboratory setting. Obviously, that is too intensive to be addressed manually. Other options must be identified.

Following the same line of reasoning (injecting additional fluid to maintain desired pressure), something must take the place of the unfortunate laboratory assistant equipped with a syringe. While injecting additional fluid into the bioreactor system at short intervals of time for potentially multiple days in a row is not reasonable for a laboratory assistant, an automated system would likely be much more suitable. If the rate of pressure drop is consistent and accurately determinable, a syringe pump may prove effective. Syringe pumps can be fitted with a wide array of syringes – including those with the luer lock design. A barbed male luer lock fitting could be attached to the syringe. Next, a length of silicone tubing could be attached to the barb. The free end of the tubing would then be placed on the center barb of a three-way barbed fitting. The bioreactor's tubing would then be cut at a specified point and the two free barbs of the fitting would be inserted to span the gap. As the system would run, it would leak at a specific rate and the syringe pump would replace the leaking fluid at the same rate. Thus, the leak would be nullified for all practical purposes and the base-line pressure would be maintained.

There would be many difficulties with using this syringe pump setup. First, an extensive battery of tests would have to be conducted to accurately characterize the cumulative leak behavior of the system. This would be both time consuming and complex. The difficulty arises from the potential variability from one system to another and the repeatability in generating the same leak rates. Even with all of the same parts, each assembly would be slightly different. Any discrepancy in the rate of fluid entering the system relative to the rate of fluid exiting the system would result in a drift in pressure over time. Also, over extended periods of time, the leaks may not be constant. The rate of leakage could change over time and the syringe pump would not be able to adjust to the varying rates. Another issue is the scalability of the syringe pump. Tying multiple systems into a single pump may deplete resources too quickly and cause problems. Plus, syringe pumps are expensive and take up a lot of space. Finally, in order to ensure agreement between injection and loss rates, auto regulation utilizing pressure feedback could be incorporated in the design but would be extremely expensive.

Therefore, the syringe pump and similar fluid injection techniques would not solve the problem, but they are an important conceptual step in the correct direction.

Overall, upon completion of the pressure loss experiments described in this chapter, it was concluded that fluid injection would not be a viable option for back-pressuring the bioreactor system. The system had a continuous leak so it would require a source of continuous supply in order to sustain consistent pressure. A number of potential alternate solutions will be explored in the next chapter.

Chapter 6: SELF-REGULATING INJECTION PROCESS

Introduction

The concept of continually replacing lost fluid from the bioreactor system by injecting a complementary volume of fluid back into the system, as discussed at the end of Chapter 5, is explored further in this chapter. The syringe pump serves as an example of a "blind injection process." For the purposes of the studies in this chapter, blind injection processes are defined as techniques or methodologies of injecting fluid into a system at a constant or preset rate without any negative feedback or self-regulating properties. Blind injection processes are not suitable for the highly variable demands of the new or old bioreactor systems that would be necessary for maintaining desired base-line pressures. For example, if a system variably leaks at rate within a range of 1 to 2 ml/hr and an attached syringe pump attempts to compensate for the loss at a constant rate of 2 ml/hr, the syringe pump will outpace the leak. This would ultimately result in an increasing system pressure which would have its own associated risks and problems.

Therefore, it is the goal of this chapter to introduce the idea of self-regulating injection processes as potential solutions to the back-pressuring objective and to develop an initial design to assess the feasibility of implementing such methods. Self-regulating injection processes, within the context of this study, are the opposite of their blind counterparts. They will be loosely defined as any technique or methodology of injecting fluid into a system at a rate adjusted by negative feedback or some other regulatory means. The major benefit to such systems or processes is that they "know" when to stop.

Creating a self-regulating injection process for testing was a complicated task. The basic idea and guiding principles used in this study to develop the technique will now be described. A three-way valve would be placed in line with the bioreactor. Its location with respect to the bioreactor chamber would not be important as it would pressurize the entire system homogenously. Attached to the three-way valve would be a luer lock syringe full of fluid. A custom stand would be necessary to hold the syringe perpendicular to the bioreactor tubing within the incubator, but a ring stand is sufficient for bench top testing. A weight could then be attached to the plunger of the syringe to apply a constant force to the system. This would, in theory, continually replace the lost fluid and prevent pressure loss as a constant force is applied. This method could be applied in conjunction with manual pressure injection to sustain the backpressure or as an independent means of obtaining the desired pressure levels. The implementation and evaluation of this concept and theory are described in the subsequent sections of this chapter.

Methods and Materials

In order to test this method, a simplified bioreactor system was used once again. The system utilized one 6 inch length of silicone tubing, one two-way valve, one three-way valve, a pressure transducer, two luer lock syringes, a ring stand, a plastic CD case, and a partially filled 32 oz plastic bottle of water. One end of the 6 inch length of tubing was connected to the pressure transducer using luer lock fittings. The free end of tubing was then connected to one end of the straight-through flow path of a three-way valve with a barbed female luer lock fitting. The free end of the pressure transducer was fitted with a two-way valve. Then, a luer lock syringe was filled with water and attached to the vertically oriented “T” branch connection of the three-way valve – this vertical branch remained closed initially. A second luer lock syringe filled with water was connected to the two-way valve on the pressure transducer and fluid was injected into the system and allowed to vent through the open end of the three-way valve until the system was completely filled with fluid and no air bubbles could be seen. The luer lock syringe attached to the two-way valve on the transducer was then removed, and both the two-way and three-way valves were closed to prevent fluid loss from the system. The three-way valve was closed by shutting off the outlet port of the valve – simultaneously opening the path connecting the tubing to the luer lock syringe. A ring stand was used to secure the syringe attached to the three-way valve into a vertical position. The plastic CD case was balanced atop of the syringe plunger, and the partially fill plastic bottle was balanced upon the CD case. This set up is illustrated in Figure 6.1.

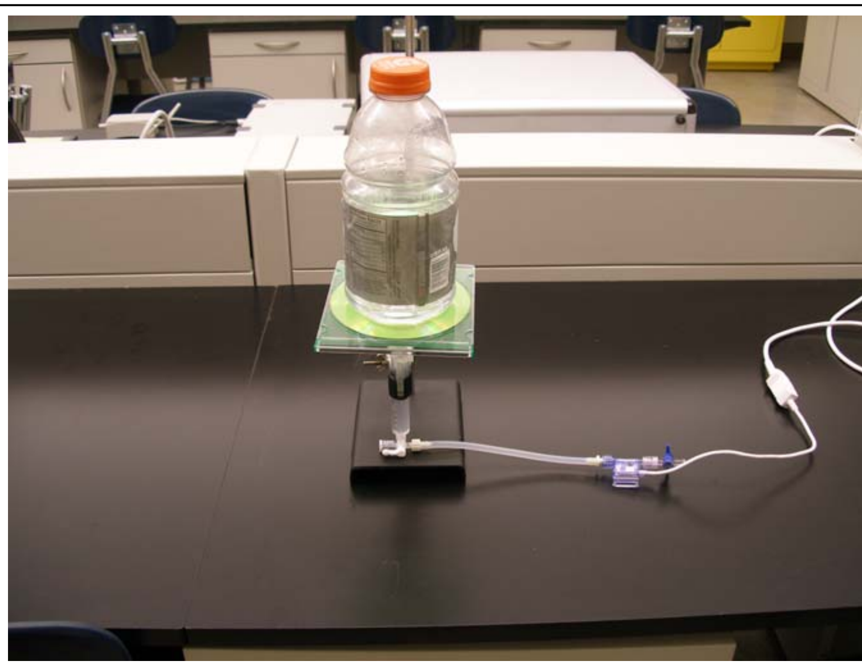

Figure 6.1 Weighted syringe assembly used to backpressure system.

Initially, a force was first applied by hand to the syringe plunger to see how well the system responded to force without the use of the partially filled plastic bottle and CD case. Next, the 32 oz bottle was used as a weight to impart constant force upon the plunger in the setup described and illustrated above. The sturdy CD case was balanced on top of the plunger to augment the surface upon which the water bottle rested. 33.8 oz of fluid is equal to 1 liter. 1 liter of water weighs approximately 2.2 lbs. Accordingly, the 32 oz plastic bottle could provide the range and magnitude of force necessary to produce the ideal backpressures based on Equation 6.1 below.

$$P = F/A \qquad \text{(Eqn. 6.1)}$$

The outcome of this test set up was assessed by pressure recordings taken by the data acquisition equipment.

Results

By applying more or less force by hand, desired pressures were easily reached. In addition, by monitoring the system pressure recorded by the data acquisition software, a relatively constant pressure was maintained. However, applying force to the system using the 32 oz plastic bottle was far less effective. The pressure readings (illustrated in Figure 6.2) show a trend of decreasing rates of pressure loss over time. Nonetheless, the system failed to generate a constant pressure. A complete set of data is provided in Appendix A. The average rate of pressure loss was 0.318 mmHg/min based solely on initial and final pressure measurements. Interestingly, the rate of pressure loss was far from constant. At the beginning of the experiment, measurements recorded by the data acquisition equipment indicated an approximate 4 mmHg/min rate of pressure loss. Measurements recorded at the end of the test showed rates of pressure loss as low as 0.11 mmHg/min. While the trend of the data points did indicate a decreasing rate of pressure over time, it did not occur constantly. Rates continually fluctuated in magnitude every couple of minutes – increasing and decreasing at times. The presence of a small air bubble was also noticed after 30 minutes of the experiment. No quantitative measurements were taken to characterize the size or volume of the trapped gas, but it was periodically checked over the course of the study and found to decrease over time. The bubble began as approximately half of the size of an eraser on the end of a pencil. At the end of the experiment, only 1/6 of its original size appeared to remain.

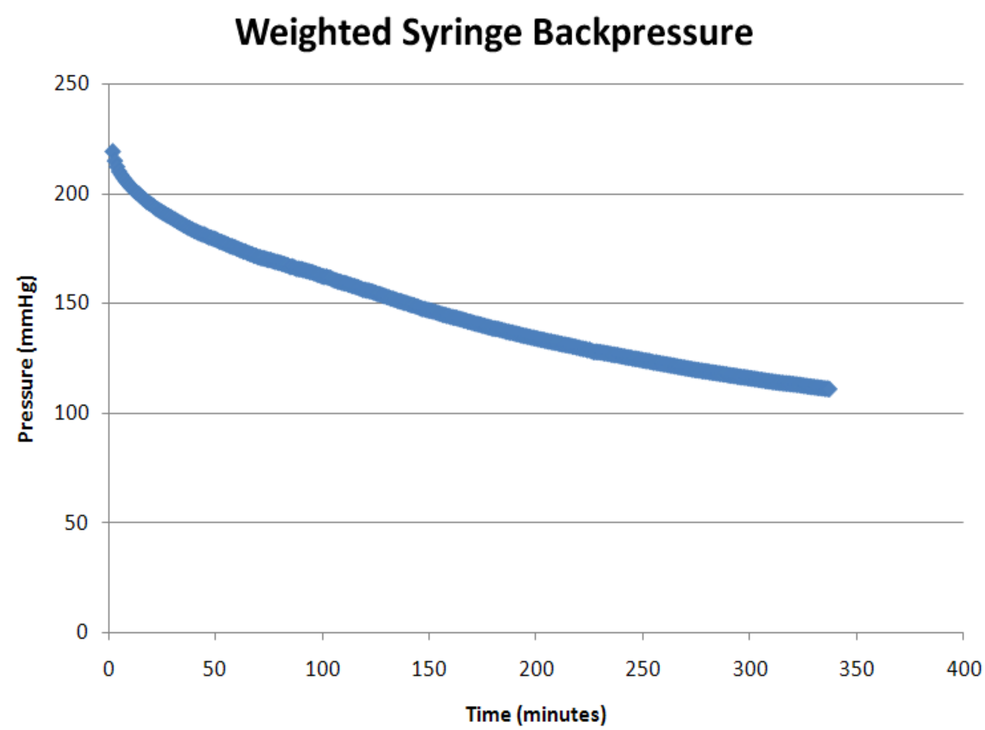

Figure 6.2 Measured backpressure over time – induced by weighted syringe setup.

Discussion and Conclusion

The constant backpressure measured and maintained in the manual force trial was not reproduced with the weighted syringe. By pushing down the plunger manually, it was possible to minimize the resistance stemming from physical and frictional forces generated by the seal of the syringe plunger and wall. Monitoring the pressure measurements recorded by the data acquisition equipment, a constant pressure of 100 mmHg was maintained with relative ease. Even so, it was difficult to estimate the amount of force that was applied by hand while the manual test was conducted. It was also difficult to determine whether or not a constant force was actually applied. As a result, the manual test was only beneficial in crudely testing the theory and providing results that supported further inquiry and evaluation with the weighted syringe. Accordingly, the next step involved conducting the trial with an object of mass rather than by hand, as illustrated in Figures 6.1 and 6.3.

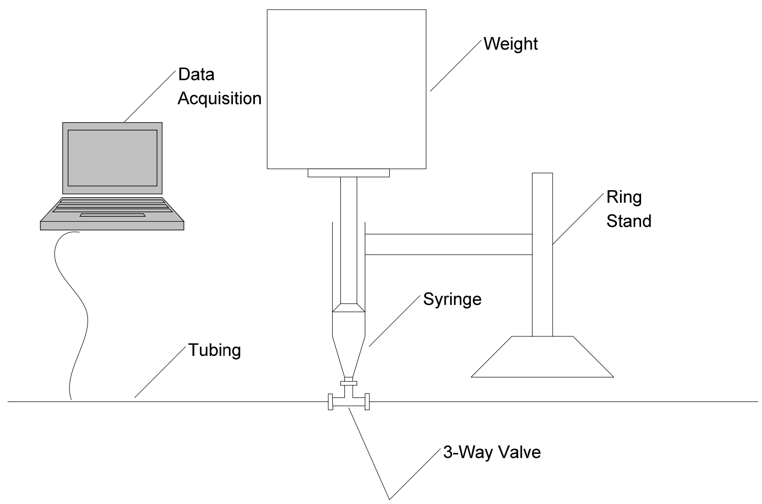

Figure 6.3 Modeled weighted syringe backpressure setup illustrating the general system parts and their role in establishing a constant applied force.

The failure to generate a constant backpressure appeared to be partially due to applying an insufficient weight to entirely overcome the plunger’s frictional forces. This conclusion was based on the varying rates of pressure loss observed during the trial. As illustrated by the graph in Figure 6.2, the rate of pressure loss started out high. The rate appeared to decrease as the study reached the 100 minute mark, and then the rate slowly increased and decreased once more. As a result, it was hypothesized that the water bottle may have been too light to prevent the plunger from sticking to the syringe walls.

Fluctuation between static and kinetic coefficients of friction could be a reasonable explanation for the varying rates of pressure loss.

Equation 6.1 was used to help resolve the question of whether or not frictional forces played a role in the pressure measurements. The necessary force required to generate a desired pressure within the system can be estimated using Equation 6.1.Based on the equation, pressure (P) is equal to the force (F) applied to a specified area (A). It would require 0.49 lbs to generate a pressure of 100 mmHg using a 10 ml syringe with a plunger surface area of 0.256 in^2 (Syringe Selection Guide, www.harvardapparatus.com). Approximately half of the 32 oz bottle was filled with water before it was placed on the syringe. Based on the previously mentioned conversions, the bottle likely weighed a little over a 1 lb. Equation 6.1 would estimate the pressure generated to be slightly over 200 mmHg. This is close to the actual initial pressure measurements recorded from the trial by the data acquisition equipment. Since the calculation does not take into consideration the frictional forces counteracting the force supplied by the water bottle, the pressure recorded by the pressure transducer would be expected to yield a lower pressure than the estimate. The pressure recorded by the data acquisition equipment was nearly equal if not slightly larger than the estimate. As a result, the frictional forces were likely negligible and contributed very little to the pressure variations. Accordingly, other potential explanations were considered.

Another potential source of pressure loss was the diminishing height of the water column within the syringe used in the study. Even so, this factor should have had only a minimal impact upon the system pressure. Considering the setup in Figure 6.3, the pressure at the base of the three-way valve depends upon two contributing factors. The first factor is the water column height within the syringe. The 10 ml syringe can only hold 3-4 inches worth of fluid. 1 inch of water roughly correlates to 1.85 mmHg. Therefore, the 4 inch column of water accounted for about 7.4 mmHg of the total backpressure. The second factor is the 1 lb. weight resting on the syringe plunger that accounts for nearly 200 mmHg. The weight of the water within the syringe is only a fraction (3.7%) of the applied force attributed to the weight. Accordingly, as the fluid is lost from the system and the water column weight diminishes, variation in pressure should be minimal. As a result, this implicates another factor as being the source of the majority of the lost pressure during the experiment.

Previously, in Chapter 5, various factors influencing pressure drop were explored. One such factor was permeability of tubing. The silicone tubing is permeable in order to facilitate CO2 exchange with the environment within the incubator. Accordingly, it was hypothesized that trapped air bubbles could pose an issue for back-pressuring the bioreactor system as the gasses may be forced out of the tubing – lowering system volume and decreasing pressure. The way this system was designed, however, it would compensate for those volumetric losses. As fluid is lost from the system, a complimentary volume of fluid is passed from the syringe back into the system. The pressure lost due to the decreasing height of the water column is negligible compared to the pressure provided by the weight. During this study, a small air bubble was noted at the end of the luer lock fitting connected to the three-way valve (illustrated in Figure 6.4). The size of the trapped pocket of gas significantly decreased over time. Interestingly, the rate of pressure loss also significantly decreased over the course of the study. It would be easy to jump to the conclusion that the two factors were interrelated. However, it was only a coincidence. As just mentioned, fluid volume lost from the system is readily compensated for by system design. More importantly, the magnitude of the pressure lost from the system (almost 100 mmHg) is equivalent to a 54 inch tall water column. The weighted syringe only operates with a 4 inch water column. Accordingly, for this much pressure to be lost, there would have to have been no fluid left in the syringe. Oddly enough, by the end of the study, the syringe experienced no measurable loss of fluid. This left one additional solution.

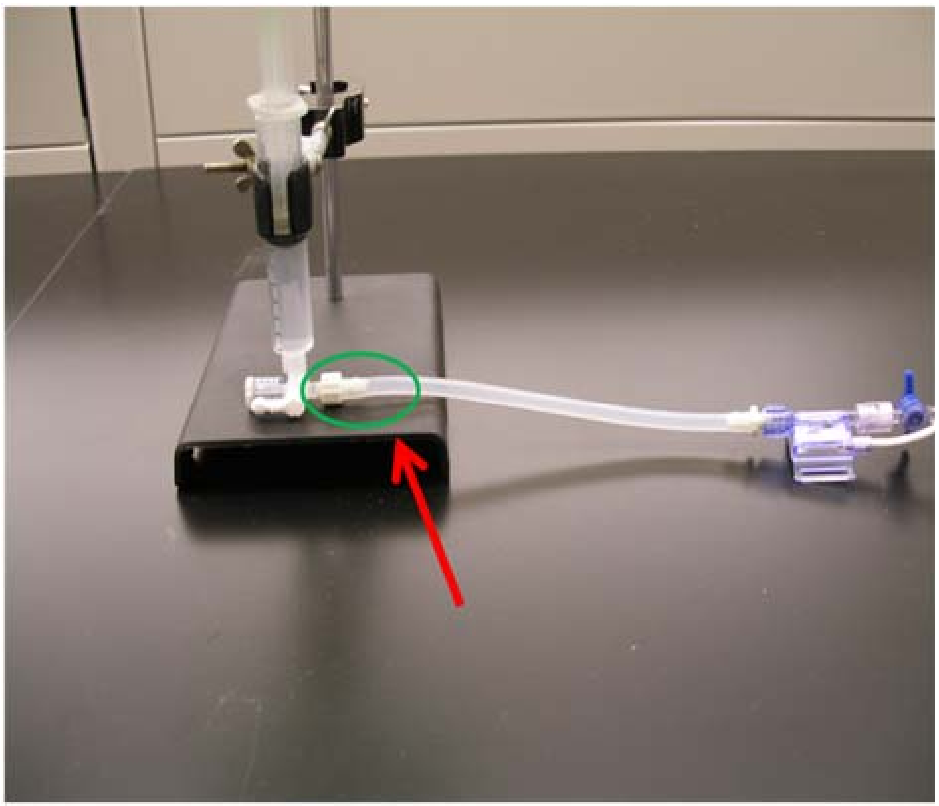

Figure 6.4 Observational evaluation of trapped bubble volume over course of study. The trapped gas appeared to permeate through the tubing.

The force applied to the syringe was inconsistent. The reason why there was such a large variation in pressure but no change in system volume was because over time less and less force was applied to the syringe plunger. One may wonder how this was possible, but it became clear when evaluating the methods used to assemble the system. The weighted syringe system is one large balancing act. The fluid-filled syringe was placed on end and held upright by the ring stand. A platform (the CD case) was balanced upon the plunger, and a weight (the bottle of water) was balanced upon the platform. Any slight misalignment would cause the precariously unstable plunger stem to lean or flex. The more fluid contained within the syringe, the less stable the extruding plunger would become. As the system leans, the full force of the weight no longer directly acts upon the fluid within the syringe. This ultimately resulted in reduced pressure levels. If the system were allowed to have continued to operate indefinitely, it would have likely fallen over or found a resting spot.

Based on the previous analysis of applied forces and potential factors influencing pressure loss, more care must be taken in future work when considering how forces are applied to the system. The weighted syringe method was a step in the right direction, but it had a flaw – the means by which force was applied. Other sources of force could be considered in future trials. For example, a shallow tray of water with a large surface area could be fixed above the weighted plunger. The tray would be fitted with a cylindrical port extending down toward the weight atop the plunger. The weight itself would have a second plunger attached to its top. The plunger would fit into the tube above connected to the tray so that it would be flush with the flat surface (Figure 6.5). The necessary steps to properly secure and align the weight would also be accomplished using this setup.

Assuming the two plungers were centered on the weight, the cylinders that the plungers fit into act as guide rails – preventing any changes in applied forces.

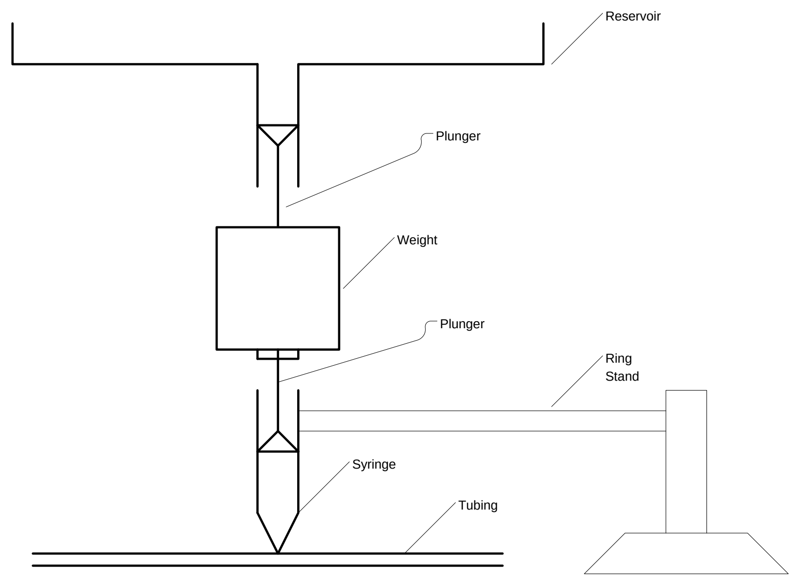

Figure 6.5 Hypothetical weighted syringe setup intended to counteract pressure loss due to water column height reduction in the syringe. The setup also secures the applied weight in place.

As the plunger falls and fluid is pressed out of the syringe below, water from the tray refills the cylinder above. The cylinder would be proportional to the syringe. As the syringe loses fluid, the cylinder gains the same amount and keeps the force constant. This would maintain a constant pressure based on the weight of the object attached to the plungers. Even though the water column only accounts for a fraction of the overall force, a highly accurate system is desired. The problem is that the pressure would be constant only as long as there is water left in the syringe. Once the water is gone, the fluid bleeding out of the system would no longer be replenished. The other problem is the amount of surface area required by the tray. The larger the tray is, the shallower the water will be above the plunger. This is important because that level will drop to compensate for the lowering plunger. The smaller that height is, the less weight variation that will occur. Another option would be to use a syringe pump, but that requires knowing the rate at which the system leaks. As discussed previously, the systems do not leak at the same rates all the time. Even if the average rate of leakage for one system could be accurately determined, no two systems will be the same. It would be difficult to scale the method to accommodate multiple bioreactors. Refilling the weighted syringe system would also be difficult to scale to accommodate other bioreactors. This method is not practical for the application. In addition, the floating weight would probably act as a dampener and oscillate with pressure waves. A base-line pressure would be maintained, but there would be no wave to speak of.

Another problem would be scaling the setup. A stand would have to be created to support all of the weights, and weights would consume a lot of space. In addition, weights can be hard to manage – especially lifting them above head, which also poses safety concerns. A single two pound weight would not cause any laboratory assistants to worry. 32 two pound weights loaded into a fully loaded incubator would not be forgiving if they were to fall upon someone. Finally, the incubator might not be able to support all of the weight necessary to back pressure all of the bioreactors.

In summary, the work in this chapter has demonstrated that self regulating injection processes are worth pursuing for back-pressuring the bioreactor system. The specific weighted syringe technique did not work as well as intended, but provided promising data to help guide future efforts. Since the weighted syringe technique is potentially highly variable and did not perform well during the study, the specific design will no longer be pursued. Therefore, the next step was to test and develop other potential techniques for back-pressuring the bioreactor system. The following chapter will discuss one such method that was derived from one of the more fundamental principles involved in the weighted syringe technique – the water column.

Chapter 7: WATER COLUMN

Introduction

As described in the previous chapter, the next step involved developing and exploring a new methodology for back-pressuring the bioreactor system since the weighted syringe setup did not perform adequately. Therefore, the goal of this chapter was to take the information gained from previous studies to design and evaluate a new technique that could be used to accurately and consistently backpressure the system.

While working on the weighted syringe method for pressurizing the system, it was noted that the weight of the water column provided some of the pressure in the system. As stated by a law explored in fluid dynamics, pressure is equal to the density of the fluid multiplied by gravity and the height of the column of fluid plus the atmospheric pressure (Equation 7.1).

$$P = \rho g h + P_{ATM} \qquad \text{(Eqn. 7.1)}$$

Gauge pressure, or the pressure within the system independent of the atmosphere, is the only pressure measurement relevant to the bioreactor system and experimental setups used in this research. Accordingly, the atmospheric term can be dropped from the equation. This was physically accomplished during the two point calibration technique used while setting up the data acquisition equipment. With the conversion of roughly 760 mmHg equal to 101.32 kPa and the density of water at STP being 998.23 kg/m^3, it is possible to calculate the height needed to generate any desired pressure. For example, in order to generate 100 mmHg, 54 inches is required. 100 mmHg is a desirable backpressure because it will exploit the symmetry of the pressure wave generated by the peristaltic pump, and it has been shown to work in practice (11). The study described in this chapter involved a simple trial to assess the feasibility of back-pressuring the system by utilizing a water column.

Methods and Materials

The materials necessary to test the theory were few. All that was required was a 50 ml reservoir, a 54 inch length of silicone tubing, a pressure transducer (calibrated before use), a short 1 inch length of tubing, a barbed luer lock fitting, a luer lock cap, and the data acquisition software. To conduct the experiment, the reservoir was first filled with water. The cap was then screwed on. This cap had two panel mounts attached to it. On one panel mount the 1 inch length of silicone tubing was attached along with the barbed luer lock fitting. The luer lock fitting was then capped off. This essentially plugged one of the panel mounts.

The following steps were performed laying the materials horizontally flat against the laboratory countertop to prevent water from spilling, as illustrated in Figure 7.1. Using a luer style syringe, enough fluid was injected into the 54 inch length of tubing until it was full of water and contained no air bubbles. The 54 inch length of tubing was then attached to the remaining panel mount. At the base of the long length of tubing, the pressure transducer was connected. This required additional luer lock fittings in order to make the connection.

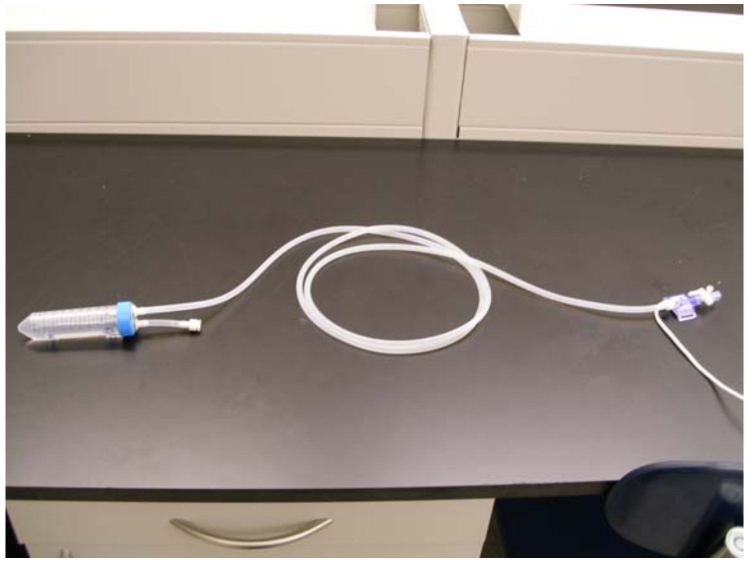

Figure 7.1 The horizontal assembly of the water column system.

The whole system was then inverted in order to generate an elevated pressure at the location of the pressure transducer, as illustrated in Figures 7.2 and 7.3. The reservoir was held using a ring stand placed on top of the laboratory countertop. The data acquisition software was then turned on to record live data.

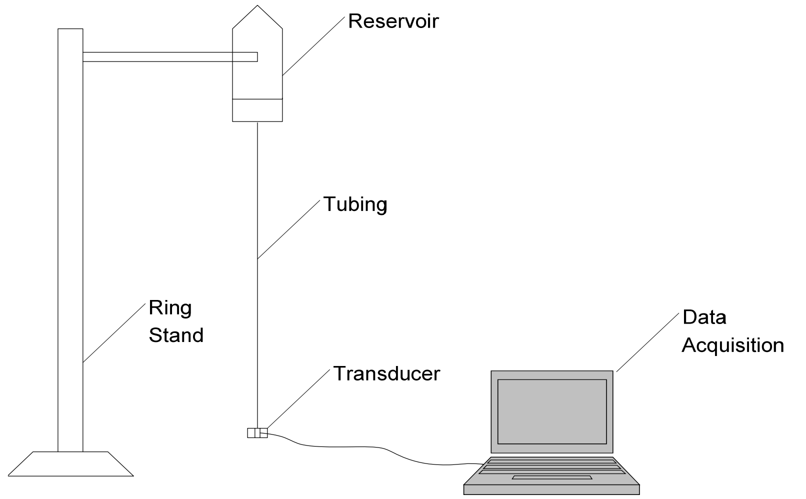

Figure 7.2 Water column backpressure setup with 50 ml reservoir.

Figure 7.3 An example of the water column setup used to backpressure the system.

Results

The pressure measured using the equipment was greater than 100 mmHg, but this was because of the additional fluid within the reservoir that effectively increased the water column height. The pressure remained constant for nearly an hour before 2 mmHg was lost. The system was left in place for 6 hours. When checked after 6 hours, the pressure had decreased an additional 12 mmHg. Over the duration of the study, the fluid level within the reservoir was also monitored. While there were no significant changes in fluid height, the level in the reservoir had dropped slightly. Table 7.1 contains pressure measurements and characteristic parameters obtained during the first part of the trial.

Table 7.1 2 hour 30 minute backpressure test with 50 ml reservoir.

Start	100.98 mmHg
End	96.53 mmHg
Total Drop	4.45 mmHg
Average	0.029 mmHg/min
Hourly Rate	1.78 mmHg/hr

Discussion and Conclusion

The 2 mmHg pressure drop described above was attributed to factors identified in the earlier statistical experiments dedicated to quantitative diagnostics of fluid leaks. Specifically, the tubing/barb interface (Chapter 5) and permeability of the silicone tubing (Chapter 6) contribute to losses in pressure. In this vertically oriented configuration, however, only the connections at the transducer were exposed to pressures approaching 100mmHg and the overall system volume was greater than that of the “large” statistical trial by roughly a factor of three. In the statistical experiment, the entire length of tubing experienced a uniform pressure that was measured by the pressure transducer. The uniform pressure is based on the level (horizontal) orientation of the experimental setups. Technically, there will be a small variation in pressure based on the fluid at the top of the cross section of tubing versus the bottom. However, the small diameter of the tubing limits the contribution of fluid level height to pressure variation in the vertical plane (Figure 7.4) to the point where it can be considered negligible. As a result of the uniform force distribution of the entirely horizontal system setup, all fittings and connections were exposed to these same forces. This is in contrast to the inverted, vertical system that would have one fitting at 100 mmHg while another is at 0 mmHg.

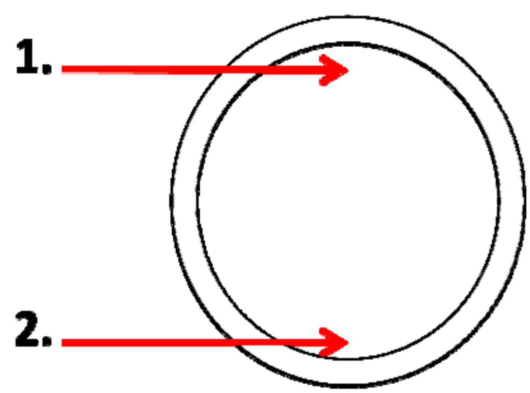

Figure 7.4 An illustration of the negligible height difference within a cross-section of tubing. Arrows indicate the height differential between the fluid at the top and bottom of the tubing cross-section that would lead to a pressure difference between points 1 and 2 based on $P = \rho g h$.

It was interesting to note that the pressure dropped roughly 12 mmHg over 6 hours, but the fluid level remained relatively constant. This was an odd result. A 12 mmHg decrease in pressure should correspond to roughly 6.48 inches of fluid loss within the setup. There is a discrepancy between the recorded measurement and the physical condition of the system at the end of the trial. While it remains unclear why this problem occurred, a possible explanation may be found in an old party trick. The trick involves a straw and a glass of water. Water is sucked up into the straw and the same end is quickly plugged by the person's thumb. The straw can then be removed from the glass of water without the water falling out of the straw. Perhaps, this setup is analogous to a large plugged straw. The inverted reservoir is sealed and as any fluid is lost at the base of the water column attached to the transducer a vacuum is formed within the reservoir. The vacuum generated imparts a force upon the water that counteracts the force of gravity that also acts upon the water. Accordingly, the fluid level may not have changed as much as the pressure did.

In spite of the lack of fluid loss per pressure drop, the results from this study were a major step in the right direction. The rate of pressure decline decreased to 1 mmHg per 30 minutes. This is much closer to the goal of constant pressure, but still excessive over a 24 hour period--too much to be considered successful. Water or media would have to be added to the systems periodically throughout the day to maintain desired levels. Maintaining the fluid level would ensure consistent system base-line pressure. Therefore, the next step was to modify the water column design in order to significantly reduce the rate at which the water level would decrease.

Chapter 8: WATER COLUMN II

Introduction

The study performed in Chapter 7 found the water column technique to significantly reduce the rate of pressure loss that occurred in the back pressured system. While it represented a significant improvement in system design, it still fell short of accomplishing a constant or even relatively consistent back pressure that could be used in the laboratory setting. A loss of 1 mmHg every 30 minutes would still require around-the-clock supervision and maintenance by laboratory assistants. As a result, this chapter focuses on improving upon the original water column design. The experiment in Chapter 7 utilized a 50 ml conical tube as a reservoir. The reservoir has a particularly small cross-sectional area. Accordingly, a relatively small loss in system volume correlates to a significant drop in fluid level height. An example would be filling a 1 ml micropipette from the same 50 ml reservoir. The fluid taken from the 50 ml reservoir will fill a 10 inch long 1 ml pipette while barely dropping the fluid level height of the reservoir. Accordingly, modifying the reservoir to reduce the drop in fluid level height was the focus of this chapter.

One potential solution was to increase the reservoir volume and specifically the cross-sectional area, so that the rate of pressure loss would be decreased. Since the columnar height dictates the base-line pressure, reducing the rate at which the water level drops would make the pressure drop at a slower rate. This chapter will further explore this concept.

Methods and Materials

The same setup was used as in water column experiment I, except a new reservoir replaced the original. A T225 culture flask was used as the new reservoir in hopes of significantly delaying the loss of pressure, and the culture flask was specifically chosen to be able to simply exchange caps between the two reservoirs. The threads for the T225 culture flask and 50 ml conical tube were different and such a trade was not possible. However, the T225 culture flask cap has a cloth filter concentrically aligned with the end of the cap, and a barbed luer lock fitting was pressed through the filter without significantly compromising the seal. While this may have leaked slightly, the effect of the larger reservoir should still be noticeable. The reservoir was filled with fluid, capped, and connected to the 54 inch length of tubing still connected to the data acquisition software. Once again the system is inverted and positioned using the ring stand clamp (Figure 8.1 and 8.2).

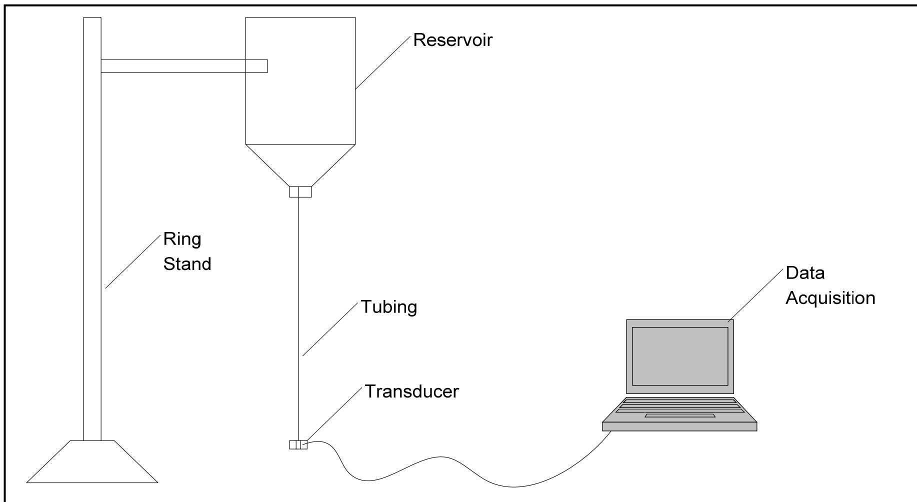

Figure 8.1 Water column backpressure setup with T225 culture flask reservoir.

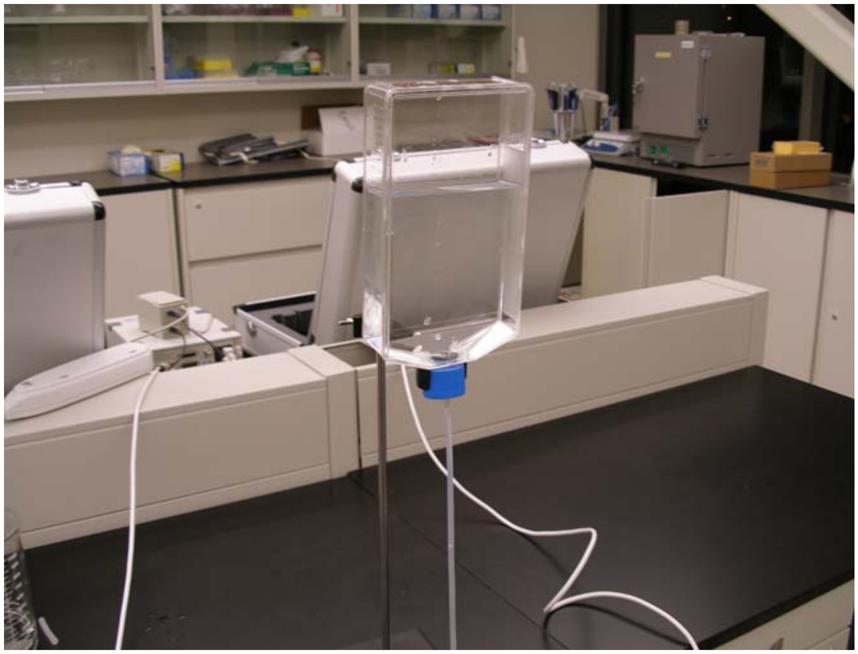

Figure 8.2 Water column test using larger reservoir.

Results

Surprisingly, no leaks were seen at the point where the barbed luer lock fitting was pressed through the filter. The 0.22 μm filter is intended to prevent bacteria from entering the culture flask, but over the course of the trial it seemed to be fine enough to prevent water from seeping through as well. At the end of the test it was a little damp, but no leaks were noted. The first pressure drop was noticed at 2 hours when 1 mmHg was lost. The system was allowed to hang for an additional 6 hours and 3 additional mmHg were lost. Table 8.1 contains characteristic pressure measurements recorded during part of the trial.

Table 8.1 250 ml reservoir water column trial conducted over 2 hours and 30 minutes.

Start	103.90 mmHg
End	102.47 mmHg
Total Drop	1.43 mmHg
Average	0.0095 mmHg/min
Hourly Rate	0.57 mmHg/hr

Discussion and Conclusion

The results from this study were quite interesting. Just by a visual comparison, the flask had roughly four times the surface area as the original reservoir. Based on the data, it is apparent that the rate of pressure drop turned out to be four times greater with the smaller flask than with the larger. While these tests have been more qualitative in nature, the empirical evidence shows promise. Although the larger reservoir provided a slower rate of pressure drop, losing 1 mmHg every couple hours is still not desirable.

While improving the consistency of the pressure, large reservoirs consume a lot of space within the incubator and require more media. In addition, there is the added challenge of developing mounting hardware in order to suspend these containers within the incubator. The real issue, however, is that the leak rate is still too great. Losing 1 mmHg every 2 hours will result in a 12 mmHg drop in 1 day. This would require that the system receive daily attention, which is both expensive and time consuming. Also, a 10 mmHg drop can be the difference between modeling slight hypertension at 130/90 mmHg and normal conditions at 120/80 mmHg. A more accurate and consistent model can and must be developed.

Chapter 9: WATER COLUMN III

Introduction

Changes over time in the height of the fluid level within the column have been a consistent problem in each of the water column based backpressure systems. The water levels in the reservoirs have been decreasing as a result of leaks in the trial evaluations of simplified bioreactors. It was also noted during regular laboratory trials that the bioreactors used to cultivate BVMs in the incubator tend to run low on media over time. Whether this was due to leaks, some evaporative process, tissue metabolizing the nutrients, or fluid redistribution within the system, it was not known. These losses posed a concern when utilizing an independently attached water column to provide a backpressure for the system. “Independently attached” is intended to describe an addition to the bioreactor perfusion loop that does not have fluid directly flowing through it (Figure 9.1). Fluid losses or redistribution would cause decreasing fluid level heights within the attached reservoir and result in an inconsistent backpressure. The water column trials up to this point had all been based on static conditions for the system. Static conditions were useful for reducing variables influencing pressure drop and as well as accurately monitoring pressure levels. Dynamic conditions, applied correctly, may resolve the issue and this chapter will explore the effects.

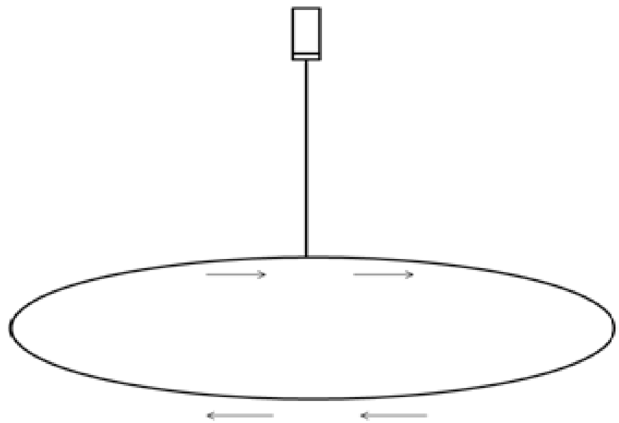

Figure 9.1 Independently attached water column. Arrows indicate the directional flow of fluid within the bioreactor system. Flow within the loop does not enter the water column.

Theoretically, the problem of decreasing fluid level heights within the water column could be solved by simply establishing a flow-through reservoir with the requisite height differential relative to the bioreactor chamber – thus creating a perfused water column (Figure 9.2). A dynamic system will continually perfuse fluid through the tubing of the bioreactor system. Consequently, there would always be fluid in the tubing connecting the bioreactor chamber to the reservoir inlet as long as the fluid level in the reservoir remained above the outlet to the supply line (the tubing section that connects the reservoir to the pump inlet). In this type of system, the backpressure will still be provided by the water column height of the silicone tubing connecting to the reservoir inlet. Any fluid that is lost from the system during use would result in a decrease in the fluid level height in the reservoir and not the water column leading to it. The amount of fluid in the reservoir would not have any impact upon the column of fluid feeding into the reservoir through the inlet side tube. This essentially creates a water column between the separated bioreactor chamber and reservoir that never loses its height. With this in mind, the water column backpressure technique explored in the previous studies will be applied to a running bioreactor system in its entirety.

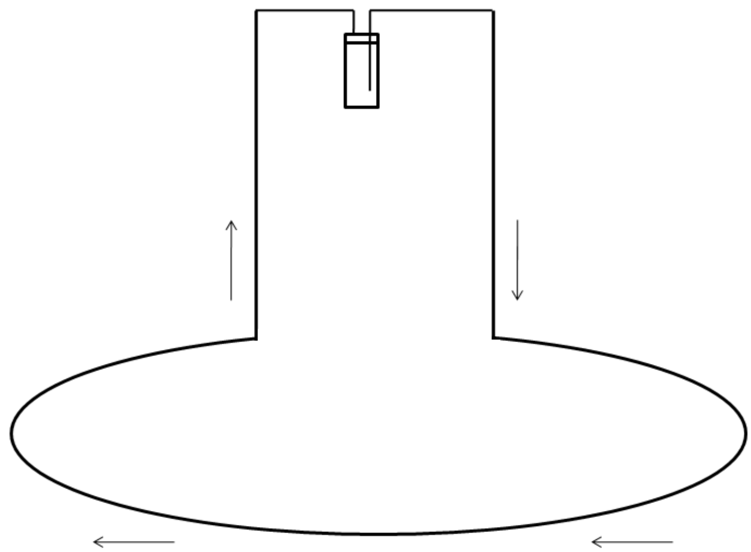

Figure 9.2 Perfused water column. The reservoir is no longer independently attached to the perfusing loop of the system.

Methods and Materials

In order to conduct this experiment, numerous materials were required. To begin, the setup included a 50 ml reservoir, Masterflex peristaltic pump with a 3 roller pump head, and a Lock N Lock bioreactor chamber. The reservoir was suspended using a ring stand affixed with a test-tube clamp. The cap of the reservoir had two panel mount luer lock fittings inserted in it. One was the inlet (system return) and the other was the outlet (system supply). The outlet fitting had a length of silicone tubing attached that extended down deep into the reservoir and was well below the surface level of the liquid within it. This configuration was intended to compensate for fluid losses and subsequent drops in fluid levels within the reservoir. If the fluid level would drop below the tubing extension, fluid would cease to be returned to the pump and the system would run dry. A length of silicone tubing was used to connect the outlet side of the reservoir to the platinum cured pump tubing secured to the pump. Male and female barbed luer lock fittings were used to connect the silicone tubing to the pump tubing. The free end of the pump tubing was fitted with an additional male and female barbed luer lock fitting combination and attached to another length of silicone tubing. This silicone tubing was then connected to the inlet side of the bioreactor chamber. The inlet side of the bioreactor chamber had a single panel mount barb connection for attaching the tubing. The outlet side of the bioreactor chamber had two panel mounts with short lengths of tubing joining at a “Y” fitting connection. The center-line luminal fitting had a two-way valve placed between the “Y” fitting and the bioreactor chamber wall. A second valve was located just after the combined branch of the “Y” fitting. A long length of silicone tubing was connected to the second valve using another luer lock fitting. The free end of the tubing was then connected back to the inlet fitting in the reservoir cap. The reservoir height was set at 54 inches, measured from the top of the cap to the chamber center-line. Next, a length of ePTFE was suspended within the bioreactor chamber. Once the loop had been completed, the ring stand and pump were set upon the countertop while the bioreactor chamber rested upon the floor (Figure 9.3).

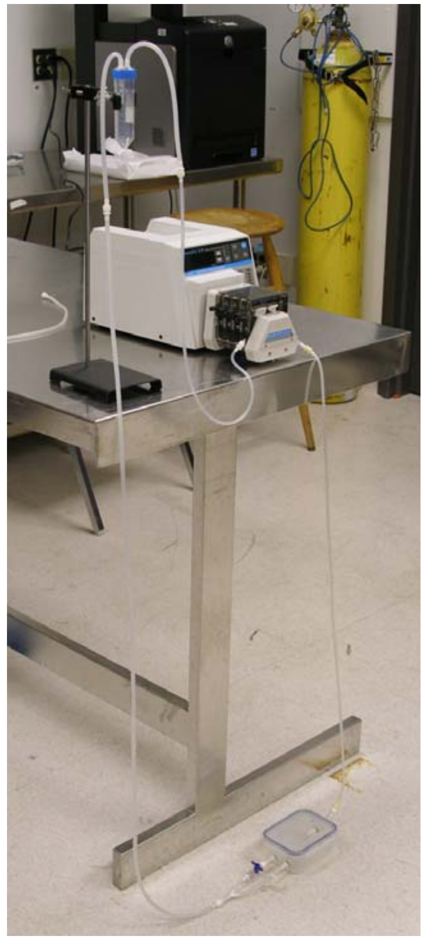

Figure 9.3 Bioreactor setup used to generate physiologic pressure waveforms.

Introducing fluid into the system required a couple of steps. Fluid was first added to the extra-luminal space of the bioreactor chamber. Water was poured into this space around the scaffold until it was up to the brim of the Lock N Lock container. The snap on lid was then secured. There were a number of ways to fill the tubing with fluid, but the easiest utilized the pump. A selected cartridge head was removed from the pump. The pump tubing was then laid across the pump head and the cartridge was reinstalled – compressing the tubing. Even with the tubing gates dropped down into place on the pump cartridge, the tubing “walked” out the side of the cartridge when the pump was turned on. It was necessary to turn the pump on at a low RPM in the clockwise direction to perfuse fluid toward inlet side of the bioreactor chamber. The pump tubing was then pulled snug in the cartridge in the direction of fluid flow so that there was no slack. This was done so that the luer lock fitting connecting the pump tubing to the silicone tubing was flush with the tubing drop gate on the cartridge. The fitting acted as a stop to prevent the tubing from being further pulled through the cartridge. The occlusion adjustment knob on the cartridge was set so that there was a 1/4 inch gap between the occlusion wedges. This had two important consequences. It set the pressure wave amplitude to 20 mmHg – which corresponded to a 40 mmHg fluctuation between the pressure wave highs and lows. It also ensured that fluid was adequately pushed through the system.

To prime the system, the cap to the reservoir was unscrewed and placed in a beaker full of water. The end of the supply tubing on the outlet of the reservoir was fully immersed. The pump was then turned on and the speed set at 45 RPM. The speed was not critical – it was dictated by the patience of the operator. A slower rate produced fewer bubbles in the line, but required more time. Once the system was full of water, it was necessary to throttle the pump on prime a few times to pass any trapped gas through the lines and out of the system. The cap was then removed from the beaker and affixed to the reservoir.

To measure the internal pressure of the system, the data acquisition equipment was tied into the system. The pressure transducer was positioned downstream of the bioreactor chamber and in horizontal alignment with the chamber. To install the transducer, the chamber outlet two-way valve was closed, and a hemostat was used to clamp off the tubing connecting to the valve. The transducer was injected with fluid to prevent the introduction of air bubbles into the system. Once the tubing and valve were sealed, they were disconnected and attached to the transducer – which bridged the gap. The reservoir, initially on a horizontal plane with the chamber, was elevated with a 54 inch offset to generate the desired 100 mmHg base-line pressure (Figure 9.3). Data was then collected over a 25 hour period running the system at 20 RPM.

The data acquisition equipment included both hardware and software. It was assembled and calibrated using the same procedure as before. A confirmation of measurement accuracy was obtained from comparing the measured pressure generated by the 54 inch water column to the estimated pressure the transducer should read based on the water column (100 mmHg). Data points were entered into the table after the experiment was concluded by scrolling through the first 72 hours of data and manually recording the pressure measurements every hour.

Results

With the initial setup completed and under static conditions, the pressure of the low point of the system (the bioreactor chamber) measured between 101-102 mmHg. As soon as the pump was started, the systolic and diastolic pressure measurements were 125/85 mmHg. Over the following 25 hours, measurements were taken continuously with the data acquisition equipment and recorded every hour. Table 9.1 displays the recorded data beginning after the first hour of operation. Figure 9.4 depicts a sample of the recorded waveform after two hours of system operation.

Table 9.1 Systolic and diastolic pressure measurements reported hourly for a 25 hour test.

hour	systolic pressure (mmHg)	diastolic pressure (mmHg)
1	120.44	80.56
2	117.94	78.19
3	116.46	76.86
4	115.79	75.89
5	114.67	75.28
6	113.39	73.90
7	112.17	72.57
8	117.12	77.27
9	115.95	76.50
10	115.03	75.33
11	114.52	75.00
12	114.06	75.18
13	113.09	75.63
14	113.75	74.10
15	113.95	74.72
16	113.80	74.31
17	113.39	75.02
18	113.29	74.26
19	112.93	74.36
20	117.12	78.19
21	116.05	77.07
22	114.72	75.99
23	113.24	76.09
24	117.73	78.44
25	116.61	77.32

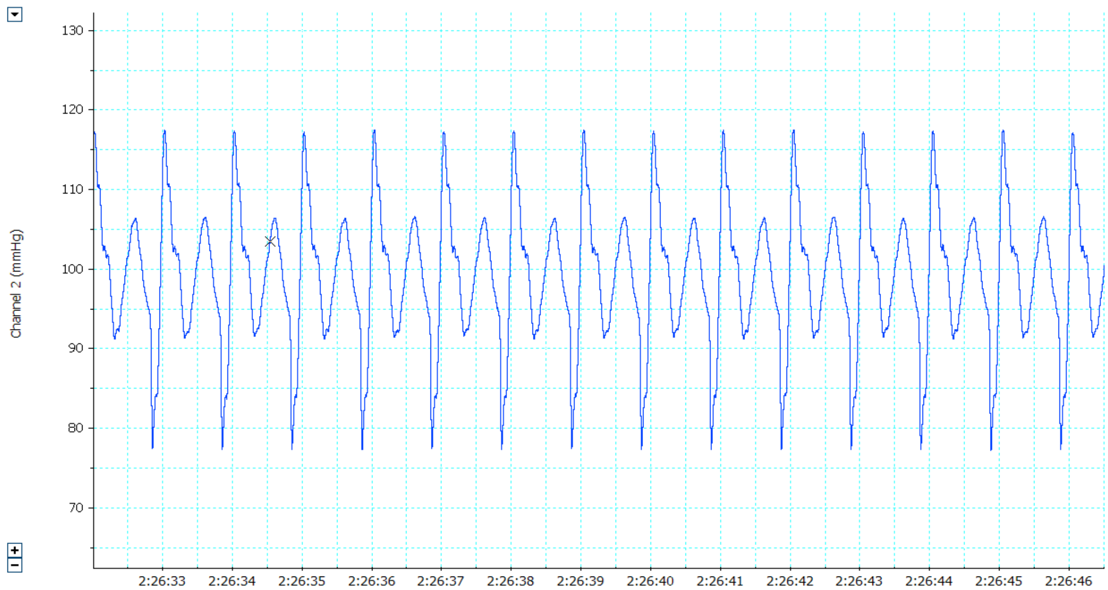

Figure 9.4 Sampled pressure waveform recorded after 2 hours of bioreactor operation.

Discussion and Conclusion

Once the 25 hour test was completed, the pump head was disassembled. Upon disassembly the pump tubing was examined. Significant deformation of the tubing had occurred. The side in contact with the cartridge head had an accumulation of black wear debris and the tube no longer retained a cylindrical shape. While this is not ideal, it is normal and should not have any effect upon the results of the measurements. No cuts or breaks were found in the tubing. In addition, the tubing is “crushed” as a part of normal peristaltic function. Accordingly, the shape (deformed or otherwise) of the tubing crushed within the pump should not significantly affect pressure or flow provided the flow volume is not substantially altered under these circumstances. However, the tubing does undergo a break-in period and this phenomenon may affect initial measurements versus data recorded at a later time.

Initial readings were high compared to the rest of the measured systolic and diastolic pressures. The pressures began at approximately 125/85 mmHg and then settled at a peak of roughly 117/78 mmHg. The drop is most likely due to the tubing breaking in over the first hours of use. The other source of variation was due to capping the reservoir. When the cap was screwed on, additional gasses were trapped within the chamber and compressed. This led to an artificial spike in pressure that could not be maintained by the system. As a result, while running the bioreactor over a period of time, any leaks present within the system decayed the magnitude of the initial pressure that was in excess of the pressure the pump and water column were able to generate. For example, the water column is capable of generating and maintaining 100 mmHg while the pump can produce a pressure wave of ± 20 mmHg. Accordingly, the system should be able to sustain 120/80 mmHg. However, the system began at 125/85 mmHg after the cap was attached to the reservoir. The additional 5 mmHg was not generated by normal system operation and, therefore, could not be maintained with the presence of leaks. Even with leaks, the system should have been able to maintain the 120/80 mmHg pressure wave.

If one looks closely at the data, there are pressure shifts that occur between hours 7 and 8, 19 and 20, as well as 23 and 24. At 7 hours and roughly 41 minutes, systolic pressure had dropped all the way to 111.76 mmHg. Over the period of 11 seconds, the systolic pressure climbed up to 116 mmHg (Figure 9.5). At 19 hours and roughly 15 minutes, systolic pressure had decreased to 112.47 mmHg. Over a period of 8 seconds, the systolic pressure climbed up to 116.30 mmHg. At 23 hours and roughly 32 minutes, the systolic pressure once again dropped and reached 112.83 mmHg. Over a period of 8 seconds, the systolic pressure climbed up to 116.36 mmHg. The amount of time leading up to the minimum pressures varied and occurred over extended periods of time. The self-regulated restoration of pressure, however, was abrupt and relatively consistent. In addition, the systolic pressures consistently oscillated between 112 mmHg and 117 mmHg. As one can see, the pressure would continue to climb after the initial drop until the systolic pressure would reach 117 mmHg. The gaps between the drops in pressure were not steady, but the pressure would fluctuate within a relatively consistent range.

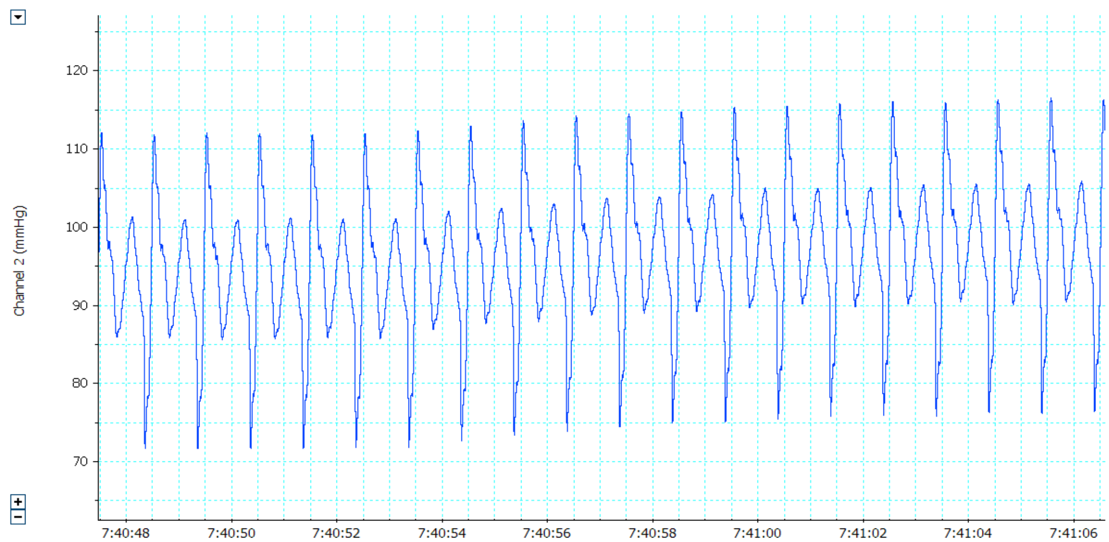

Figure 9.5 An example of the system's ability to self-regulate backpressure. Pressure decreased over a period of time until a critical point was reached where the system regained some of the initial backpressure.

Based on the results, it appears that the closed system was generating a small vacuum in the reservoir as fluid was lost from the system. The reservoir was the highest point in the system and, as a result, the location of the lowest pressure. The system has been shown to leak in the previously conducted studies – especially the Lock N Lock bioreactor chambers at pressures at 100 mmHg and above. Upon inspection of the bioreactor chamber, a water ring could be seen where it had been sitting. This was an obvious indication that fluid was lost from the system and supports the conclusion that a vacuum was generated in the reservoir – the high point of the system. Interestingly, the data also suggests that there was potentially a limit to the magnitude of the vacuum that the reservoir could withstand without compromising any of the seals. Once this point was exceeded, approximately -8 mmHg (from 120 mmHg), the panel mount seals on the cap were compromised and air was drawn back into the reservoir over an 8-10 second period until nearly the original pressure was restored. The reason the pressure does not return all the way back to 125 mmHg is because this pressure was an artificial peak caused by the capping of the reservoir, and the compromised seal became functional at a pressure slightly less than 120 mmHg. During disassembly of the reservoir, a sucking sound could be heard once the cap seal was broken.

This study confirmed earlier suspicions of the Lock N Lock bioreactor chamber's inability to withstand elevated pressures. 120 mmHg is not much greater than the reported leak pressure for the extra-luminal space of the bioreactor chamber, and it is only momentary. Since the pressure is not maintained at a constant elevated pressure, the chamber does not leak as drastically as it did during the leak pressure test. The study also confirmed that the pressure is able to diffuse beyond the scaffold barrier into the surrounding extra-luminal space. While the leak was not catastrophic, it did exist. The Lock N Lock bioreactor chambers could be successfully used with this experimental setup in future laboratory trials, but they generate vacuums within the system and a small mess. This issue qualifies the use the newly designed bioreactor chamber in the future studies performed for this project. Even so, an additional study will be performed with the Lock N Lock bioreactor chamber in order to confirm observations made in this study.

The leaks in the system would cause a vacuum because the system is run by a positive displacement pump. The pump will push the same amount of fluid that it pulls. If there is a disparity between what the pump draws and pushes, the system will either pressurize or depressurize. When the operations are balanced, the pressure is constant. During the experiment the pump was pulling a certain amount of fluid from the reservoir, but that same amount was not being returned to the reservoir because of the slight leaks downstream of the pump. Since the bioreactor used in the experiment was a closed loop system, the loss of fluid created a vacuum.

Overall, this experiment had some flaws, but they can be corrected. The significant positive result of this study was that it functioned within a consistent pressure range and was relatively insensitive to the minor leaks that have proven critical flaws in the other configurations. The system was insensitive to the leaks in the sense that pressure was lost over a period of hours rather than minutes and fell out of the systolic range of 117 mmHg to 112 mmHg. Based on this, it is worthwhile to continue using this basic backpressure technique but to proceed with a few modifications. Since the sealed reservoir was where the vacuum was generated, venting the reservoir would prevent the phenomena from occurring. More testing is required to validate the results obtained in this study. While leaks were found and a drop in pressure was detected, this was only a single study. Another similar test will be performed to confirm the results. The next experiment performed in the following chapter will include an improved bioreactor design that will specifically eliminate the capping pressurization and reevaluate the potential vacuum formation.

Chapter 10: WATER COLUMN IV

Introduction

Based on the observation of pressure fluctuations in the previous study and the suspicions of vacuum formation, the experiment needed to be repeated to verify the accuracy of data obtained. The goal of this study was to recreate the fluctuating pressures observed in Chapter 9 and to obtain additional information to validate and better characterize the hypothesis of vacuum formation. Effort can then be dedicated to assessing the frequency and severity of the problem. If observations made during the last test were anomalies, it would not make sense to modify the system to compensate for them. Accurately defining system behavior now will lead to more efficient solutions later. Therefore, the focus of this study is to confirm the existence of vacuum formation within the bioreactor system and to better characterize its occurrence.

To accomplish this, the system was only slightly modified. It was important that the system from the last study was not drastically changed as it may have had an impact on the results that were obtained and phenomena that were observed. However, the last study began with artificially elevated pressures due to capping the reservoir. The initial spike in pressure may have caused instability within the system that may have impacted the rate at which the vacuum formation occurred. With this in mind, only a small modification was implemented in this study to the reservoir. This modification allowed the reservoir to be manually vented in order to prevent elevated pressure generation during the reservoir capping process.

Materials and Methods

The materials and protocol were the same as described in the previous round of testing. The only differences were that this test was conducted for a slightly longer period of time (31 hours instead of 25), and that a three-way valve was installed at the inlet to the reservoir in order to vent any pressure generated while capping the reservoir (Figure 10.1). As was just mentioned, the bioreactor system was assembled using the same method as mentioned as in Chapter 9, with one small change. Before the cap was attached to the reservoir, the 3 way valve attached to the reservoir's inlet was opened to the atmosphere by closing the inlet port of the valve. This configuration allowed excess gasses to escape the reservoir and vent to the open air during capping. Even though the Lock N Lock bioreactor chamber had shown a propensity for leaking at elevated pressures, it was used again to assess its operation a second time and to gather more data to evaluate its ability to function under operating conditions. In addition, during the previous study the leaking bioreactor was implicated in vacuum formation. Since this study was focusing on better characterizing and understanding the potential vacuum, it was important to keep the system the same and not remove the potential cause. The pressure transducer used to collect data from the system was calibrated and zeroed before use. Data points were entered into the table after the experiment was concluded by scrolling through the 31 hours of data and manually recording the pressure measurements every hour.

Figure 10.1 System modification to allow venting of initial pressurization from capping process.

Results

The initial pressure without the pump running was 102 mmHg. Once the pump was started, pressure was measured at 121.46/82.99 mmHg. The pressure recorded over the following 31 hours is listed below in Table 10.1. A sample of the generated pressure waveform measured at 31 hours is illustrated in Figure 10.2.

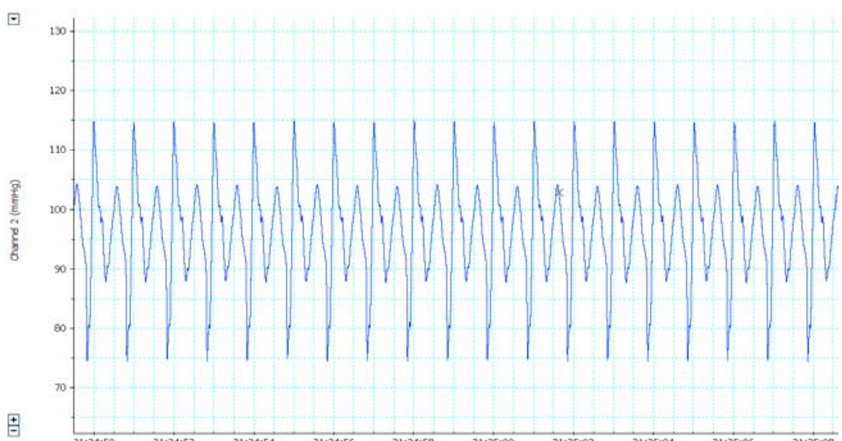

Figure 10.2 An example of the generated pressure waveform after 31 hours of bioreactor operation.

From Table 10.1 it can be seen that the pressure remained much more consistent over time. The pressure varied very little, only a fraction of 1 mmHg, in the first 14 hours of system operation. However, after this point, pressure appeared to decrease and then fluctuate between highs and lows (Figure10.2).

Table 10.1 Systolic and diastolic pressures reported hourly for a 31 hour test.

Hour	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)
1	122.18	83.25
2	121.98	83.15
3	122.38	82.89
4	122.49	83.20
5	122.54	83.50
6	122.08	83.96
7	122.69	84.07
8	122.74	83.09
9	123.25	83.20
10	122.38	83.71
11	122.90	84.58
12	122.64	84.52
13	122.38	85.34
14	122.49	85.90
15	121.52	86.98
16	121.21	87.49
17	121.26	85.00
18	118.30	78.19
19	114.87	75.12
20	117.99	78.50
21	114.11	77.42
22	119.73	83.66
23	120.34	84.52
24	120.19	84.93
25	120.19	85.04
26	120.90	85.80
27	124.12	85.14
28	124.73	85.19
29	121.00	82.79
30	118.30	78.96
31	115.69	76.25

Discussion and Conclusion

By removing the initial peak pressure generated during the capping process, the system modification and change in protocol seem to have had an effect on the stability of the pressure within the system. The pressure remained relatively constant for the first 14 hours of testing. After 14 hours, a vacuum began forming until pressure was once again reestablished. After the first fluctuation, two more fluctuations occurred. One occurred almost immediately after the pressure was restored. The next began to occur at the end of the test.

It appears that the frequency of fluctuations was a result of the system self-correcting. The previous test began with a pressure that was elevated slightly above the vented state. Accordingly, pressure quickly dropped in the first hour and a new set point was reached. Since the system adjusts and corrects as a function of fluid loss and the integrity of the seals on the reservoir, there is some variation in the period over which it may occur. This test started with the air space in the reservoir vented to atmosphere and the initial system pressure was subsequently lower than in the previous test. The cap was put in place with the valve open so that pressure would not build as a result of the capping process. Once in place, however, the valve was closed to the atmosphere so that the fluid within the system would circulate. As a result, the pressure built up over the following 9 hours after testing began. There are three potential explanations for the buildup of pressure within the system. The first potential cause is the presence of a few trapped air bubbles within the water column portion of the bioreactor system. These bubbles would detract from the pressure generated within the bioreactor chamber because they reduce the weight of the water column since air is less dense than water. After a few hours of operation, these bubbles may have been cleared from the line and the homogenous water column exerted a greater pressure within the bioreactor. Another potential explanation is that numerous air bubbles trapped throughout the bioreactor loop were dislodged over time and compressed within the bioreactor reservoir. The other explanation is that the pump pulled air into the system through a compromised seal or cracked tubing somewhere between the pump and the reservoir. As the trial progressed, the pressure fluctuations were likely due to system self-correction. There are system limitations to maximum and minimum pressures. Once these limits were surpassed, the system automatically restored resting conditions to some degree. The amount of correction that the system has to go through might result in over correction and affect the rate at which the fluctuation occurs as well as the magnitude. It is more likely, based on the data, that once the system limits are surpassed a seal somewhere in the system (probably the reservoir) is compromised. The variability of what seal is compromised, and to what degree, affects the pressure fluctuations. The same seal might not always respond the same way to the dropping pressures.

Based on this experiment, simply venting the system a single time before running the pump is not sufficient to maintain a constant pressure throughout system operation. In addition, vacuum formation was not an anomaly. Results in this experiment were similar to those observed in the previous experiment. Accordingly, the problem associated with leaks and fluctuating pressures still existed and must be dealt with. The next study will address the problem using a permanently vented reservoir.

Chapter 11: WATER COLUMN V

Introduction

It was noted in earlier discussions of experimentation with the water column that a vacuum had been forming in the reservoir. The vacuum was likely caused by a leak within the system on the discharge side of the pump, which unbalances the relationship between fluid supplied by the reservoir and fluid returned to the reservoir. The net loss results in vacuum formation and is cumulative over time. A potential solution to this problem is to leave the reservoir vented to the open air throughout the duration of the test. This will hopefully eliminate the issue of pressure fluctuation within the system. The experiment described in this chapter implemented this potential solution. Vented reservoirs have been used in other research facilities, but with the intent of allowing gas exchange (9). Accordingly, it is feasible to use the vented reservoir during regular laboratory experimentation without compromising the sterility of the system, as long as a filter is incorporated. In this experimental study the vent was meant to serve a different purpose other than facilitating CO2 exchange with the media – it was implemented to help regulate pressure fluctuations.

Materials and Methods

The same setup protocol used in the previous experiments (Water Column III and IV) was applied to set up this experiment, except the manual vent from the previous study was not included. The reservoir was placed at the same height (54 inches), the same connections and lengths of tubing were used, and all of the fittings were the same. However, this test differed in the type of reservoir modification used. Instead of using the normal reservoir with only an inlet and outlet mounted in the cap, this reservoir had a cap with three ports (Figure 11.1). Using this slightly modified setup, procedures to measure and record pressures over an 18 hour time span were carried out as described in the previous chapter. Data points were entered into the table after the experiment was concluded by scrolling through the 18 hours of data and manually recording the pressure measurements every hour.

Figure 11.1 Reservoir cap with 3 ports.

The reservoir cap had an inlet and outlet just like the previous system, but there was an additional panel mount that vented directly to the atmosphere. In this study, the newly designed bioreactor chamber was also implemented instead of the Lock N Lock. The Lock N Lock had proven inefficient at operating under dynamic conditions with elevated pressures. In addition, it was no longer necessary to characterize the formation of vacuums within the system. This study focused on eliminating pressure fluctuation rather than creating it. Measurements were taken continuously over an 18 hour period using the calibrated ADI pressure transducer and data acquisition equipment.

Results

The beginning base-line pressure of the static system was 101 mmHg. Once the peristaltic pump had been started, the pressure wave oscillated between 119.22 and 80.64 mmHg. Table 11.1 below contains the data recorded over the following 18 hours. Once the pump had been stopped at the end of the experiment, the base-line pressure read between 101 and 102 mmHg. In addition, the new bioreactor did not show any signs of leakage. Figure 11.2 illustrates the pressure waveform generated by the peristaltic pump and is characteristic of all pressure waveforms the system generated under these applied conditions.

Table 11.1 Systolic and diastolic pressures reported hourly for an 18 hour test.

hour	systolic pressure (mmHg)	diastolic pressure (mmHg)
1	120.14	80.23
2	119.93	80.13
3	120.39	79.72
4	121.16	78.85
5	121.11	78.75
6	121.77	78.44
7	121.57	78.14
8	121.98	77.93
9	122.54	78.44
10	123.61	78.39
11	123.51	78.29
12	123.81	77.88
13	123.2	77.42
14	123.15	77.83
15	123.87	77.12
16	123.81	76.91
17	124.17	76.96
18	123.81	77.12

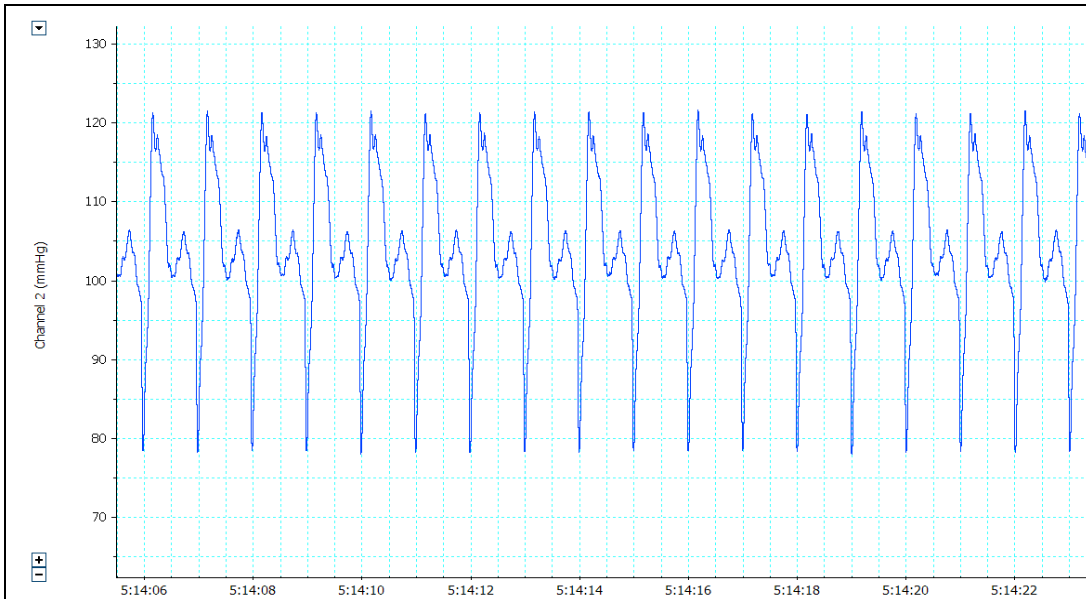

Figure 11.2 Recorded pressure wave during 18 hour test.

Discussion and Conclusion

Based on the data generated from this test, it appeared that the fluctuating pressures had been nearly eliminated. There was some small variation within the data, but not nearly as much as in previous studies. Although this was an encouraging result, the pressure wave at the end of the study was not the same as the beginning pressure wave. The float or variation in measurement was likely due to wear-related changes in the pump tubing over time. This assumption is based upon the changes in the pressure waveform over time. Instead of the entire pressure wave shifting up or down, as a result of pressurization or vacuum formation, the amplitude of the wave increased. The increase in pressure wave amplitude resulted in increased systolic pressures and decreased diastolic pressures, but maintained the same average pressure (approximately 100 mmHg). A simultaneous increase in systolic pressure and decrease in diastolic pressure cannot be accounted for by the previously explored pressure fluctuation mechanisms – especially considering the included vented reservoir. A shift in pressure wave magnitude is substantially different from an increase in wave amplitude. Just as a vacuum can be generated in the reservoir as fluid leaks out on the discharge side of the pump, pressure can be generated within the reservoir by leaks on the suction side of the pump. A leak on the suction side of the pump would result in air being pulled and forced into the system. System temperature may also play a role in pressure increase. Thermal expansion of the fluid within a closed system would result in an increased volume and pressure. However, the system was no longer closed. As a result, volume changes no longer resulted in pressure changes. Accordingly, thermal expansion or leaks on the suction side of the pump could not generate increased pressures in the system. Clearly, the increased pressure wave amplitude must have been due to some physical property of the system changing. Since the wave grew over time, the increase in amplitude was likely due to the degradation of the pump tubing over time. Nonetheless, a number of potential explanations were explored before dismantling the system.

The pump was stopped and restarted to see if any change in pressure would occur due to any potential operational or flow related factors. If a change was noted, attention could be focused on analyzing the potential factors and their relation to pressure. However, no changes occurred. After the pump had been stopped and restarted, a couple of bubbles were noticed within the tubing line in a few places throughout the system. Pockets of gas can alter pressure readings within the system since air is a different density and exhibits different fluid properties. For example, air is compressible while fluid is not. The presence of a less dense and more compressible fluid within the system should not result in elevated pressures. In fact, since air is less dense and compressible, an increased volume of air within the system lines would result in lower pressures and smaller pressure wave amplitudes. To remove the bubbles from the system, the system was primed by increasing the RPM of the pump. Priming the pump removed most of the bubbles and the system returned to 121/80 mmHg. After priming the pump a second time, a small leak was noticed. Therefore, moving the air bubbles out of the system was not the likely cause of the pressure wave amplitude reduction. In previous experiments, worn pump tubing was found to lose its shape and accumulate silicone debris as black or gray deposits on the outer tubing wall. Priming the pump may have cleared or shifted such an accumulation of debris to a position where it may not have impacted tubing occlusion any longer. This is plausible considering the tubing was so worn out that the second time the system was primed the tubing began to leak.

Removing the cartridge head from the pump revealed that the platinum cured tubing had been significantly degraded over the course of the experiment. The tubing had large debris deposits and a large crack in the longitudinal direction. New pump tubing had not been used in this experiment because the tubing is rated to endure many cycles and repeated use. It is easy to imagine the condition of the pump tubing affecting the pressure waves, especially since adjusting the occlusion of the cartridge played a role in determining the amplitude of the pressure wave when initially assembling the system. Cole Parmer had been contacted in regard to the condition of the pump tubing. The accumulation of worn debris on the outer surface of the tubing was considered normal with extended use. Future trials will be conducted with new pump tubing.

Another potential source of variation could be due to temperature. Fluid expands as it is heated. The cyclic nature of the peristaltic pump may elevate the temperature of the fluid slightly. An increase in temperature would cause the fluid to expand over time and result in higher pressures and larger amplitudes. Even if the pump itself did not change the temperature of the system significantly, ambient temperature of the room may fluctuate and have the same end effect. Even if this was the case, the vented reservoir should counteract these effects. The end goal is to run the bioreactor within an incubator. The incubator is maintained at 37°C. Since this is a constant temperature, there should not be any volume fluctuations due to the environmental temperature. However, the 37°C temperature of the incubator is elevated compared to standard temperature and pressure (STP) conditions that designate 25°C as room temperature. If bioreactor materials assembled outside the incubator are near room temperature, thermal expansion will likely occur when the materials heat up to 37°C inside the incubator. The expansion may be minimal, but the vented reservoir would compensate for any volumetric changes.

Overall, the new bioreactor chamber proved to be durable and able to withstand the environmental conditions of the trial. Whether the increased stabilization of pressures was attributable to the addition of the new bioreactor or the vented reservoir, it was not specifically deduced. It was likely some combination of the two factors, but the vented reservoir was able to counteract volumetric fluctuations whereas the bioreactor chamber simply attempted to prevent them. At the end of the trial, the bioreactor chamber did not show any signs of leakage and there were no wet spots on the floor where the bioreactor had been sitting.

In the future, it will be important to use new pump tubing for experiments designed for prolonged periods of time in order to ensure similar testing conditions and comparable results. The short test demonstrated that the vented reservoir was effective in significantly reducing pressure fluctuation. A fluctuation of only a few mmHg is acceptable and attributable to nuisance factors. These results warranted an extended trial to assess the stability of the system under more realistic laboratory conditions.

Chapter 12: 72 HOUR TEST

Introduction

The study conducted in Chapter 11 evaluated the efficacy of the permanently vented reservoir in preventing pressure fluctuations within the bioreactor system. The results from the study indicated that the system modification was successful in eliminating pressure oscillations within the bioreactor. However, the results also indicated that the pressure wave amplitude increased over time. Increasing pressure wave amplitude is undesirable, but it was attributed to the degradation of old pump tubing used in the setup. Accordingly, the undesirable results were independent of the system modification to the reservoir and could hypothetically be remedied with the inclusion of new pump tubing during system assembly. Therefore, the bioreactor system with the elevated and vented reservoir was ready for a long-term trial.

The purpose of the tests presented in this chapter was to run an extended trial to assess the stability and durability of the final backpressure system design. The longer the system can run without assistance or intervention, the more practical and successful the design is. As a minimum, 72 hours of operation without intervention is desirable. This would allow the system to run over a standard 3 day cell culture period without requiring a laboratory assistant to make additional adjustments. Intervention would be defined by a laboratory assistant needing to make any adjustment to the system to maintain the desired pressure levels. The experiment in this chapter was therefore conducted over three days and the system was periodically checked to confirm proper function.

Materials and Methods

The system was set up using the same protocol described in Water Column Test V. The permanently vented reservoir and newly developed bioreactor were utilized in this system. The pressure transducer was calibrated using two point calibration and zeroed before use. Data points were entered into the table after the experiment was concluded by scrolling through the data and manually recording the pressure measurements every hour. The test was designed to run for 72 hours.

Results:

Results for this study were only recorded for 13 hours before the test was aborted due to abnormal data collection. The base-line pressure of the system before testing was measured at 100 mmHg. The final base-line pressure of the system was 112 mmHg.. The measurements are listed below in Table 12.1. Once the pressure transducer was removed from the bioreactor system, the data acquisition equipment measured 12 mmHg under atmospheric pressure.

Table 12.1 Systolic and diastolic pressures reported hourly for a test terminated after 13 hours.

hour	systolic pressure (mmHg)	diastolic pressure (mmHg)
1	120	80
3	122	82
5	125	86
7	127	88
9	129	90
11	131	91
13	132	92

Discussion and Conclusion

The test was terminated early, after only 13 hours, because of the inexplicable increasing trend in pressure. Data during this period was not consistent with previous studies and an explanation for the deviation could not be determined without inspection of the system. Before taking the system apart, potential sources of error were considered. These sources included increasing fluid or gas volume, a shrinking system, the development of flow restriction within the system, or pressure transducer drift. Increasing fluid or gas volume was dismissed based on environmental conditions. The trials were conducted within a laboratory maintained at a relatively constant temperature. Therefore, the gasses and fluids should not have been heating up and expanding within the system. In addition, the reservoir was vented and should have accommodated any volumetric changes of system constituents. One might propose that the extra-luminal space within the bioreactor chamber may have acted as a closed system, and that an increase in volume within this space may have exerted additional forces upon the system. However, previous tests showed that the permeability of the ePTFE scaffolding allows for trans-mural diffusion of pressure. Accordingly, the extra-luminal space would be at equilibrium with the luminal region of the bioreactor chamber and the vented reservoir would still account for such volumetric changes. The same reasoning can be applied to system shrinkage. If for any reason the tubing, reservoir, or bioreactor chamber constricted, the vented reservoir would have allowed fluid levels to freely adapt, thereby preventing system pressurization.

The most probable source of variation was concluded to be the pressure transducer itself. To inspect the transducer without altering the rest of the system and risking loss of potential evidence, a hemostat was used to clamp off the tubing downstream of the pressure transducer and the two-way valve upstream was closed. The transducer was then removed and inspected. The data acquisition equipment was left running to assess potential measurement drifts during inspection. Oddly enough, once the sensor was taken out of the system it read 12.32 mmHg. The transducer was zeroed before it had been inserted into the system. Logically, once the transducer was removed from the system it should have again read zero. During this trial the transducer did not maintain the zero calibration. Water was tapped out of the transducer and dried. The pressure transducer was then checked using the pressure cuff, and the pressure cuff was inflated until it read 100 mmHg. LabChart 7 was used to record data simultaneously. When the pressure cuff read 100 mmHg, LabChart measured 112 mmHg. The transducer was zeroed using the bridge pod amplifier and then connected to the pressure cuff and inflated until the gauge read 100 mmHg. The pressure cuff gauge and data acquisition software measurements were viewed simultaneously. The transducer measurement indicated by the data acquisition software reflected that of the gauge. The gauge measured 100 mmHg while the software measured approximately 99.8 mmHg. It was therefore safe to conclude that the transducer was still calibrated correctly. Water was tapped out of the pressure transducer because the transducer was calibrated dry and liquid must not be introduced into the pressure cuff. The sensor was reattached to the system using the luer lock connections. The hemostat was removed, the two-way valve was opened, and the pressure was rechecked. The pressure then read 95 mmHg, which was to be expected given the drop in the 54 inch water column height and a couple of trapped air bubbles – artifacts from removing and reattaching the transducer. The system was then primed and the pressure returned to 100 mmHg. This effectively confirmed the drift in transducer readings. It had been previously calculated that the 54 inch water column height would generate approximately 100 mmHg. If the pressure transducer did not drift, dialing down the bridge pod amplifier would have resulted in a pressure reading of 88 mmHg from the water column.

The pressure transducer drifted over the course of the trial for some undetermined reason. It was odd that the measurements would shift. A new transducer was therefore used in subsequents tests and the 72 hour test was attempted once again.

Chapter 13: 72 HOUR TEST II

Introduction:

Chapter 12 described a failed attempt at conducting a 72 hour test. Over the course of the 13 hours of operation, the data acquisition software recorded an increasing pressure over time. The pressure began at 120/80 mmHg and ended at 132/92 mmHg – an average increase of 12 mmHg. Upon system inspection and disassembly, it was concluded that the pressure transducer readings drifted and were not indicative of the true bioreactor pressures. This was attributed to a potentially faulty pressure transducer.

Once again, an extended assessment of the back pressured bioreactor design was conducted. This study was a repeat of the first attempted 72 hour test and involved implementing a new pressure transducer. The goal of this study was to use the data collected from the test to determine whether further refinements of the system were necessary.

Methods and Materials:

The bioreactor design was set up the same way as in the previous study using the water column technique. This test was conducted for 72 hours. Measurements were continuously taken every minute (See Appendix A), and data was recorded into a table manually every hour. A different pressure transducer was included in this trial to see if the drifting measurements would be eliminated. The bioreactor system was periodically checked and monitored for drifting pressures throughout the duration of the trial.

Results:

The initial base-line pressure of the system was between 102 and 103 mmHg. Once the pump was started and the testing began, the pressure wave measured 121.34/82.12 mmHg. The final pressure recorded at the end of 72 hours of testing was 122/83 mmHg. The final base-line pressure was 102.52 mmHg. The data recorded over the duration of the test is listed below in Tables 13.1 and 13.2.

Figure 13.1 illustrates an example of the measured waveform recorded 15 hours into the study. In the previous experiment, the pressure drifted higher continually for 13 hours of operation. The zeroed calibration setting of the transducer was found to have shifted by 12mmHg – thus an inaccurate indication of the true pressure within the system. Even with the new transducer used in this study, a similar measurement drifting pattern occurred. Over the 72 hour period, the bioreactor was periodically checked. On 4 separate occasions a system intervention was conducted. The interventions occurred at 16, 20, 48, and 65 hours.

The initial base-line pressure measured was between 102 and 103 mmHg. This meant, with the normal protocol, the expected pressure wave would be around 122/182 mmHg. Once the pump had been started, the pressure wave measured 121.34/82.12 mmHg – close to the expected value. Over the following 16 hours, the pressures increased until reaching nearly 130 mmHg. At 16 hours the pump was shut down and a hemostat was used to clamp off the downstream tubing connecting to the pressure transducer. The two-way valve upstream of the transducer was also closed (Figure 13.2). As in previous experiments, this allowed the pressure transducer to be removed from the system without excessive fluid loss.

Table 13.1 Systolic and diastolic pressures reported hourly for 72 hours.

Hr	Systole (mmHg)	Diastole (mmHg)	Hr	Systole (mmHg)	Diastole (mmHg)	Hr	Systole (mmHg)	Diastole (mmHg)
1	122.93	83.17	25	122.47	81.92	49	123.05	82.95
2	122.68	83.14	26	122.94	82.25	50	123.38	82.42
3	123.41	86.83	27	122.56	82.61	51	122.96	83.73
4	124.53	84.33	28	122.81	82.80	52	123.54	83.16
5	125.03	87.15	29	121.85	83.98	53	123.87	82.67
6	125.32	88.45	30	123.02	84.20	54	123.76	82.74
7	126.09	87.62	31	123.57	85.54	55	123.78	82.65
8	126.14	87.66	32	124.34	84.99	56	123.98	82.48
9	126.37	89.06	33	124.98	84.35	57	123.02	83.69
10	126.36	88.07	34	125.61	84.60	58	123.01	84.15
11	126.51	87.86	35	126.07	84.05	59	122.98	84.12
12	126.73	87.52	36	126.22	83.42	60	123.80	82.68
13	127.00	87.55	37	126.19	84.63	61	123.92	82.54
14	126.88	88.14	38	127.20	85.53	62	124.31	82.35
15	127.03	86.87	39	128.85	85.86	63	124.32	83.12
*16	128.29	83.28	40	129.36	85.70	64	124.25	83.10
17	124.31	82.95	41	128.65	86.96	*65	124.11	83.41
18	126.88	81.16	42	129.98	87.19	66	122.55	82.96
19	127.45	81.66	43	129.67	88.52	67	122.21	82.76
*20	127.99	82.22	44	130.20	88.73	68	122.18	82.62
21	122.74	82.01	45	130.87	87.54	69	122.47	82.84
22	121.71	81.76	46	130.27	89.42	70	122.39	82.91
23	121.79	82.20	47	129.87	89.80	71	122.27	82.83
24	121.79	82.28	*48	122.86	83.01	72	122.33	82.91

* denotes time point of evaluation and intervention

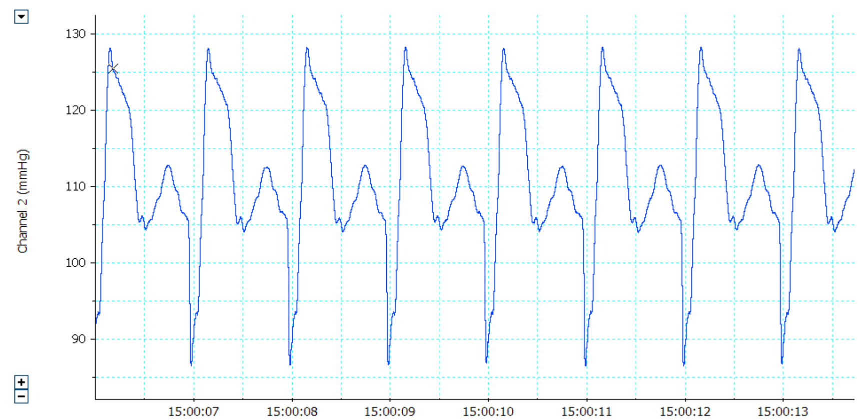

Figure 13.1 Recorded pressure waveform during 72 hour study.

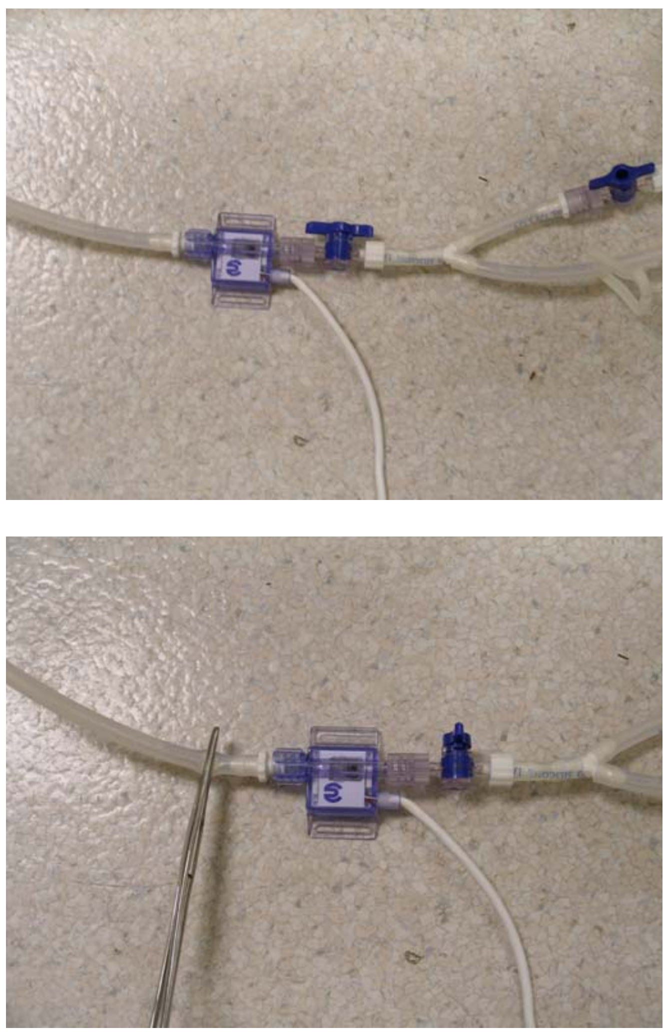

Figure 13.2 Illustration of the pressure transducer removal process during an intervention/evaluation.

Once the pressure transducer was removed, the water within the sensor was tapped out and measurements were taken using the data acquisition software. The base-line pressure read 7 mmHg instead of zero. The bridge-pod amplifier was used to zero the transducer. Calibration accuracy was confirmed using the pressure cuff by cross-checking gauge and LabChart pressure readings.

The pressure transducer was injected with fluid and reattached to the bioreactor. The hemostat was removed and the two-way valve was then opened. The pump was turned back on for a short period of time at a low RPM to minimize the introduction of air bubbles into the system and to facilitate the collection of any system bubbles in the reservoir air space. The pump was then turned off and base-line measurements were retaken. The base-line pressure had returned to 103 mmHg. The pump was turned on again and set back at the normal RPM. The resulting pressure wave measured 122/81 mmHg. The pressures concurred with the expected pressure waves based on initial and current base-line pressure readings and the test was continued.

At 20 hours, the bioreactor was checked again. Peak pressures had once again increased to 128 mmHg. Using the same method as before, the pressure transducer was removed from the system and pressure readings were checked. The transducer's atmospheric pressure read 4 mmHg. The bridge-pod amplifier was once again used to zero the transducer before reattaching it to the bioreactor. In-line base-line pressures read 103 mmHg and 122/82 mmHg once the peristaltic pump had been turned back on.

The next check was taken 28 hours later at the 48 hour mark. For roughly 10 hours after restarting the system, the systolic pressure held relatively constant around 122 mmHg. After this point in time, the pattern drift repeated. A final peak pressure of 130 mmHg was measured prior to stopping the system at hour 48 (Figure 13.3). The pressure transducer was removed and reassessed. The transducer's atmospheric pressure read 6 mmHg (Figure 13.3). The transducer was zeroed again and replaced into the system. Base-line pressure measured 103 mmHg. Once the pump was turned on, the pressure wave was roughly 122/83 mmHg. Once again, this was considered a reasonable pressure measurement for the system (based on the measured and previously calculated base-line pressures). The test was continued.

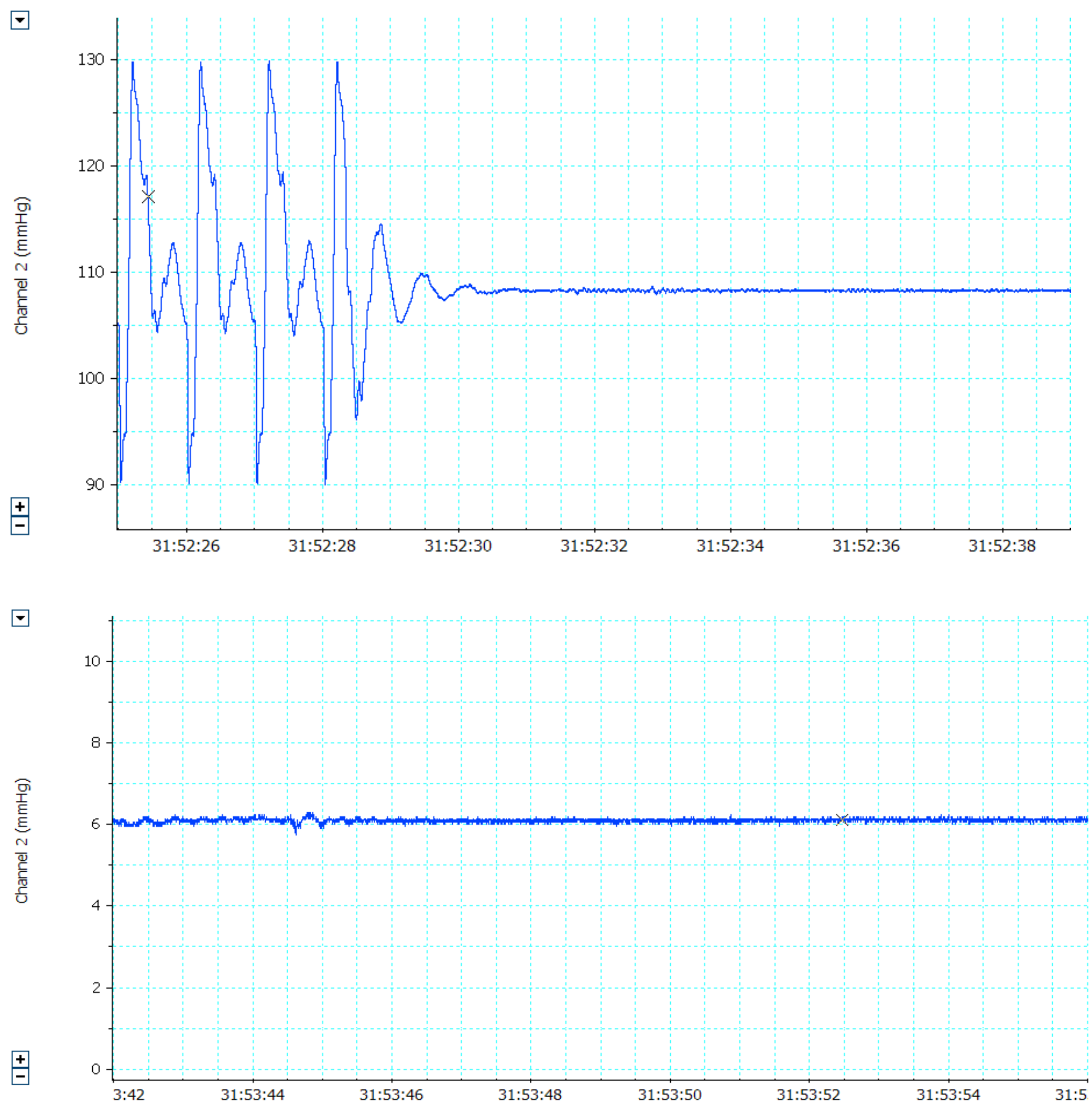

Figure 13.3 Example of drifting pressure transducer calibration. This data corresponds to the 48 hour system evaluation and intervention. Data acquisition had been temporarily shut off during the first 16 hour check, and time restarted.

The last system intervention occurred at 65 hours – a total of 17 hours later. Peak pressure readings were around 124 mmHg. This was a good sign that the pressure may have not drifted, but it was necessary to remove and evaluate the pressure transducer to ensure that this was actually true. The transducer's atmospheric pressure read 0.68

mmHg. The transducer was placed back in-line with bioreactor without recalibration. Base-line peak pressure read 103 mmHg. When the pump was turned back on, the pressure wave oscillated between 123 mmHg and 83 mmHg. There were no pressure drifts during this time period, and the pressure wave was consistent with expectations based on the resting base-line pressure of the system. The test continued until 72 hours – at which point pressure had remained relatively constant and the system was shut down.

<u>Discussion and Conclusion</u>

Changing out transducers did not solve the problem with drifting measurements. The problem most likely was equipment related. Since changing the transducer did not solve the problem, the potential source would be the bridge pod amplifier. There are a number of reasons why faulty equipment could be the source of variation rather than some other phenomenon occurring with the bioreactor setup. First of all, in previous tests, the pressure fluctuation was intermittent but cyclic in nature. The pressure would increase and then drop. The pressure variation that occurred in this test demonstrated a positive bias and correlated with a calibration shift in the transducer. Furthermore, the atmospheric venting of the reservoir would compensate for any leaks or accumulations in volume. The configuration of the water column provided a constant backpressure as long as the reservoir elevation remained unchanged. The pressure variation in the tests conducted with sealed reservoirs also had erratic fluctuations in pressure, but this was in terms of cycle initiation – once the cycle began, it followed a consistent pattern. The pressure trends in this current study varied in frequency, rate of change, magnitude of change, and direction (an increase) of change without a pattern outside of the drift itself.

In the closed system water column tests, a vacuum would be generated and the pressure would drop until a consistent minimum threshold was reached. Then the system would gain pressure within an 8-10 second period and return to close initial operating conditions, thus marking the end of the cycle. In this case, pressure just increased without reaching a threshold value.

The open system is not capable of increasing pressure over time under these conditions. Just like the vented reservoir prevented the system from generating a vacuum, it would also prevent a cumulative increase in pressure. The water column height remained unchanged; therefore the base-line pressure should have remained unchanged. Increase in pressure would require an increase in volume of fluid within a closed bioreactor system. There are two potential ways this may occur. The pump could pull in air from the outside environment, or the fluid within the system could undergo thermal expansion. If the pump had pulled in air, the gas bubbles in the line would have decreased the pressure since they are compressible. When the bubbles enter the vertical portion of the system, they displace fluid and reduce the pressure because they are less dense than the fluid that would have otherwise been there to make up the water column. As a result, measurements would initially decrease as air flowed through the lines and then increase as it accumulated in the reservoir – which the vented reservoir would not allow. Even if temperature changes were to cause fluid expansion in the system, the open system would have prevented pressure fluctuation stemming from this change as well. More importantly, a transducer that had been calibrated and zeroed before being placed in the system was removed multiple times only to find the transducer no longer read zero – when it should.

Barring the odd results provided by the data acquisition equipment, the system operated well over the extended trial. At three of the four system check-ups, equipment drift was corrected and normal pressure readings were restored. While the checks were not conducted at equally spaced intervals, they were performed throughout the duration of the test. The first check was conducted slightly after 13 hours of operation because in the previous study pressure had drifted within that time period. The next system evaluation was performed 4 hours later to assess whether or not the system correction had been maintained or if readings were continuing to drift. At each intervention, restored pressure would result in a characteristic pressure wave of approximately 122/82 mmHg. Regrettably, the skewed measurements prevented the analysis or detection of general variation trends within the data during the drifts. The test further lends to the credibility of the water column backpressure technique in that the system is durable, consistent, there are no detectable leaks or losses arising from the bioreactor chamber. Even if such variation was actually occurring, it would still be within physiologic ranges.

The water column vented reservoir technique for back-pressuring the system is an effective means of establishing desired pressures within the system for extended periods of time. While equipment problems may have skewed data during the study, the periodic system checks confirmed consistent pressure. The Chapter 11 study, which was conducted using the vented reservoir for 18 hours, illustrated how well the water column method works for back-pressuring the system. The longest duration at which a relatively constant pressure wave was maintained was 24 hours (48 through 72 hours). Even so, the pressure was consistently within the normal range for human blood pressure throughout all of the water column trials. It seems reasonable to presume that the stability of the pressure measurements observed in the 18 hour study and at the end of the 72 hour study would be characteristic of all the results in this study if the data was adjusted to compensate for the calibration drift. The restoration of expected pressures in the system was achieved several times during the 72 hour test by correcting the transducer zero calibration – no other changes were made.

Careful consideration was given the potential causes of the drifting pressure transducer measurements taken by the data acquisition equipment. As mentioned previously, the pressure transducer itself was ruled out because the drift had been observed in two trials with different transducers. The bridge pod amplifier became the next likely cause. The amplifier can be manually adjusted to amplify the recorded signal. Perhaps, manually increasing or decreasing signal amplification could impact the amplifier's sensitivity to drifting. This explanation would fit the trends in the data measurements because they increase in magnitude. The amplitude of the pressure wave remains relatively constant while the entire wave shifts upward. To test the hypothesis, trials can be conducted with the same setup from this experiment but with the bridge pod amplifier's intensity at different levels. The goal would be to observe a threshold amplifier intensity that denotes the point at which drift would no longer occur.

In summary, the inclusion of the new bioreactor chamber and water column in the bioreactor system effectively provided a means to accurately create a physiologic pressure wave and maintain it indefinitely. The drifting pressures may seem contrary to this conclusion, but the expected pressure measurements were reproduced each time the equipment had been recalibrated for the system. Now that an effective technique for back-pressuring the system has been developed, the modifications made to the system must be evaluated to ensure flow patterns have not been drastically altered. Changes in flow can potentially influence fluid velocity and wall shear stresses within the system. To estimate the generated flow conditions, computational modeling of the bioreactor system will be performed in the next chapter.

Chapter 14: INFLUENCE OF BIOREACTOR GEOMETRIES ON SCAFFOLD WALL SHEAR STRESS DISTRIBUTION

Introduction

The previous chapters explored designing a new bioreactor chamber and developing a backpressure technique in order to accurately generate physiologic pressure waves accurately and consistently. However, this work did not consider the impact the system modifications may have had on flow conditions. Therefore, the purpose of this final study was to assess the potential effect bioreactor geometries may have upon fluid flow conditions within the bioreactor chamber. As mentioned previously, tissue engineering relies on controlling physiologic conditions. There are a variety of environmental factors that play a significant role in cell proliferation, differentiation, and phenotypic expression. Such factors include temperature, CO2 levels, O2 levels, pH, pressure, growth factors, cell signaling, and mechanical stimulation (40). Cyclic strain is crucial to stimulate smooth muscle cells within the tunica media to grow, differentiate, and develop properties similar to native tissue (46; 49). The tunica intima is composed of a monolayer of endothelial cells which align, directionally, with blood flow (43, 54). Endothelial cells require stimulation from wall shear stress in order for them to adhere to the scaffold, differentiate, and align properly (37, 43, 54). Therefore, in addition to the focus on pressure described throughout this thesis, it was also necessary to consider implications of the new system for shear stress.

The newly designed bioreactor (Figure 14.1) has a couple geometries that may adversely affect the shear stress distribution within the scaffold region of the bioreactor chamber. The tubing connecting to each side of the bioreactor attaches at a swooping 90˚

bend, and the luer lock barbs supporting the scaffold are undersized (Figure 14.2). Since shear stress is crucial to endothelial cell development, it was worth investigating the influence the new bioreactor geometries may have on flow conditions in addition to the scaffold/fitting interfaces. If problems were detected, they could be eliminated and lab research could progress. This would also be the first computational model created and applied directly to the bioreactor system. Previous characterizations of flow conditions were limited to hand calculations.

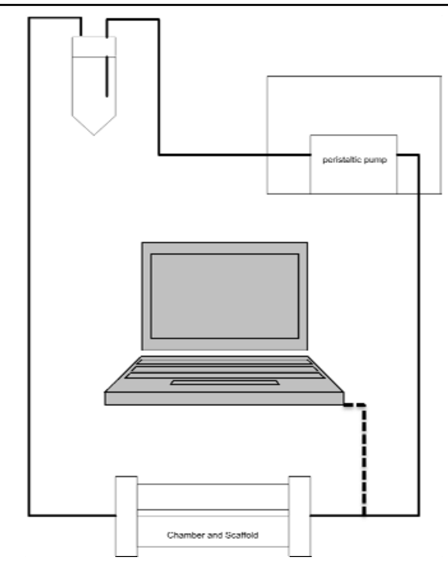

Figure 14.1 Bioreactor with reservoir, pump, and bioreactor chamber with scaffold. A height difference between the reservoir and pump relative to the bioreactor chamber creates 90˚ bends in tubing leading to scaffold.

Figure 14.2 A simplified illustration of the interface between the luer lock barb (red) and scaffold wall (black).

Methods and Materials

In order to properly model the current bioreactor system, part specification sheets were consulted and a series of measurements were taken with digital calipers for confirmation. Dimensions of interest included both the length and inner-diameter (3 x 0.4 cm) of the ePTFE scaffold, the length and inner-diameter (2.54 x 0.3 cm) of the luer lock fittings supporting the scaffold, and the inner-diameter (0.3 cm) of the silicone tubing connecting to the luer lock fittings at 90˚ angles. The scaffold length was based on the gap measurement between the tips of the inserted barbs. The surrounding bioreactor chamber was removed from the analysis because there was no trans-mural flow during normal operation. In addition, the primary focus was on the fluid dynamics within the scaffold. Using the recorded measurements, a three-dimensional model was generated using SolidWorks that accounted for the luer lock barb and scaffold diameter discrepancies as well as the angled tubing leading to the scaffold.

COMSOL software was used to perform the computational modeling necessary to characterize the velocity profiles and wall shear stresses within the system. The SolidWorks model of the abbreviated bioreactor was imported into the COMSOL program, and the physics model applied to the bioreactor design was the 3D transient incompressible Navier-Stokes model for laminar flow. Next, the density and dynamic viscosity of the fluid perfused through the system were set to 1000 kg/m^3 and 0.001 Pa·s, respectively. After completing this step, the boundary conditions were defined for the system. The inlet and outlet corresponded with the angled silicone tubing ends at each side of the abbreviated bioreactor. All of the remaining walls were defined by the "no-slip" condition. The outlet boundary condition was set so the pressure was zero. This was not indicative of the true base-line pressure within the system since it did not account for the 5333 Pa gauge pressure generated by the height differential ($P=\rho gh$) between the scaffold and the reservoir. However, this was acceptable because wall shear stress (τ_w) is dependent on volumetric flowrate (Q) in Equation 14.1 below.

$$\tau_w = (4\cdot\mu\cdot Q)/(\pi\cdot R^3) \qquad \text{(Eqn. 14.1)}$$

$$Q = (\pi\cdot R^4\cdot\Delta P)/(8\cdot\mu\cdot L) \qquad \text{(Eqn. 14.2)}$$

The volumetric flowrate is, in turn, dependent on change in pressure (ΔP) in Equation 14.2 (15). In effect, the base-line pressure would not affect velocity or wall shear stress, but the change in pressure would. The inlet boundary condition was more complex and accounted for the pulsatile nature of the bioreactor. Based on the specifications of the peristaltic pump, the volumetric flowrate for any given pump speed was known. It is typical to use a flowrate of 15 ml/min in the BVM Lab (12). Based on the Navier-Stokes equation for laminar flow in a pipe, the maximum velocity of fluid at the center of a pipe is twice the average velocity (16). Coupling this law with the flowrate equation, $Q = V_{ave}A_c$, allowed the maximum fluid velocity within the scaffold to be determined. With this information, a sine wave was manipulated to model the cyclic pulses of flow (Equation 14.3).

$$V_{wave} = V_{ave}*\sin(2\pi*t + (3\pi/2)) + Vave \qquad \text{(Eqn. 14.3)}$$

The magnitude of the wave was set to V_{ave} (.0352 m/s) and shifted up by V_{ave} so that there was no negative velocity. In previous studies, fluid perfused through the system appeared to incrementally progress. To account for this, the sine wave was not shifted up any higher than V_{ave}. This resulted in the lowest point of the wave being equal to zero and thus represented the very brief pauses that appeared to be generated by the pump. The wave velocity appropriately defines the velocities within the bioreactor since the peak velocity was equal to V_{max} (which is equal to twice V_{ave}), and mean velocity was equal to V_{ave}. The frequency of the sine wave was set at 2π to model a single pulse for every second which is equivalent to 60 pulses in a minute. The 60 pulses a minute model the healthy heart beat of a person and the estimated pulses of the peristaltic pump used at 20 RPM to generate 15 ml/min velocities (12). A phase shift ($3\pi/2$) was also added to the wave so that when time was equal to zero the velocity was also equal to zero and correlated to not having any fluid flow before the pump was turned on.

The bioreactor model was meshed once and analyzed with COMSOL. The analysis provided transient data velocity profiles, and wall shear stresses. The velocity profiles depict how the bioreactor geometries influence the fluid flow within the system and where high or low wall shear stresses may occur. The wall shear stresses are used to assess the uniformity of the stress distribution along the surface of the scaffold based on flow parameters established in previous chapters.

Results

The simulation was run for 60 seconds and data was gathered describing fluid velocity and wall shear stress within the simplified system model. While data was recorded for every 0.1 seconds, the velocity profile and wall shear stress measurements taken during peak velocity were the focus of analysis because they represented the upper limit of the bioreactor's capabilities under the preset conditions. If uniform wall shear stress was not achieved during peak velocity, it would never occur. Figure 14.3, below, illustrates the velocity profile of the fluid flow within the scaffold model at peak velocity. The figure shows a longitudinal, center-line cross section of the model.

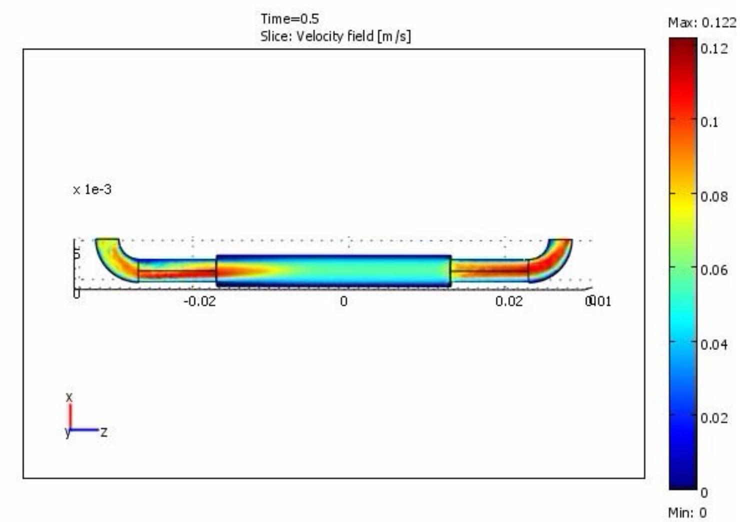

Figure 14.3 Fluid velocity profile at time 0.5 seconds.

The maximum velocity at this time point was 0.122 m/s, and the minimum velocity was zero as dictated by the no-slip boundary condition. The wall shear stress for the model was slightly more difficult to visualize as the shades of colors blend together. Figure 14.4 has two pictures illustrating the non-uniform distribution of shear stress along the wall of the scaffold. The maximum wall shear stress achieved within the scaffold region of the model was 2 dynes/cm^2 during peak velocity. There existed two transition regions where the colors changed from green to yellow and yellow to orange along the radial edge of the cross sections. This indicated that the wall shear stress increased along the length of the scaffold going from 0-2 dynes/cm^2. Approximately one third of the scaffold does not receive uniform shear stresses.

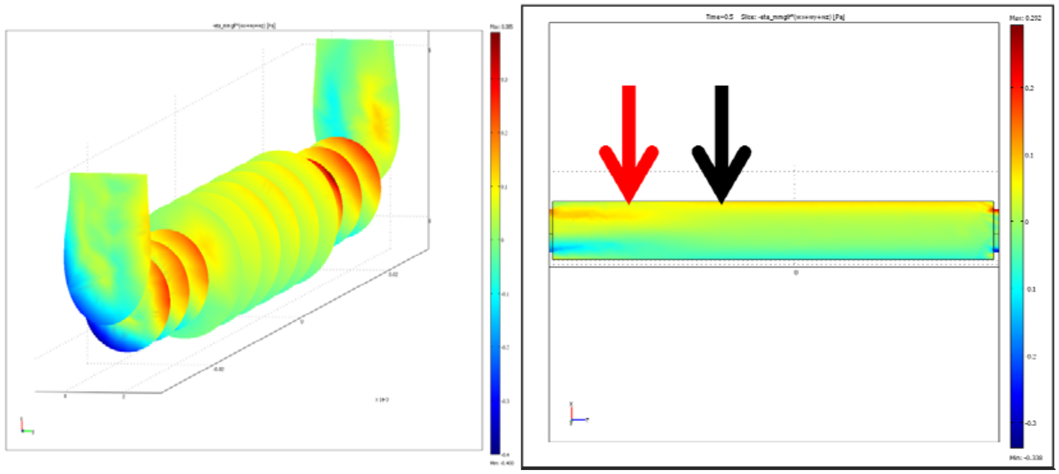

Figure 14.4 Radial cross sections of shear stress (left). Longitudinal cross section of shear stress (right). The red arrow signifies the transition from green to yellow. The black arrow signifies the transition from yellow to orange.

Discussion and Conclusion

The COMSOL computational model of the abbreviated bioreactor system appeared to be fairly accurate and a good estimate of the fluid dynamics and flow conditions within the bioreactor and corresponding small diameter arteries. The recorded maximum velocity was 12.2 cm/s (0.122 m/s). This corresponds well with measured velocities of the brachial artery found in literature (31). A study performed on the effects of age on brachial artery myogenic responses in humans recorded mean blood velocities in the brachial artery of 5.83±0.32 cm/s for young subjects and 7.44±0.74 cm/s for older subjects (31). The mean blood velocities are equivalent to an average velocity. Applying the relationship between average velocity and max velocity to their results for young subjects equates to 11.66±.64 cm/s and the recorded maximum velocity from the simulation fell within that range. In addition, as was to be expected, the calculated average velocity determined from the volumetric flowrate of 15 ml/min also fell within the range of young and old subjects at 7.06 cm/s. Accordingly, the model reasonably characterized the flow conditions within the bioreactor as well as the brachial artery.

The wall shear stresses estimated by the computational simulation showed that the inlet transition from the luer lock barb to the scaffold generated a non uniform stress distribution. The maximum measured wall stress of 2 dynes/cm^2 was low, but this was expected. The media used within the bioreactor has a low viscosity that is less than blood, and viscosity plays a significant role in determining wall shear stress as can be seen in the above equation (Eqn. 14.2). Approximately 6-10 dynes/cm^2 are required for proper endothelial cell alignment (37). Figure 14.5 (below) shows the non uniform distribution of shear stress on the scaffold wall as it relates to the velocity profile within the system.

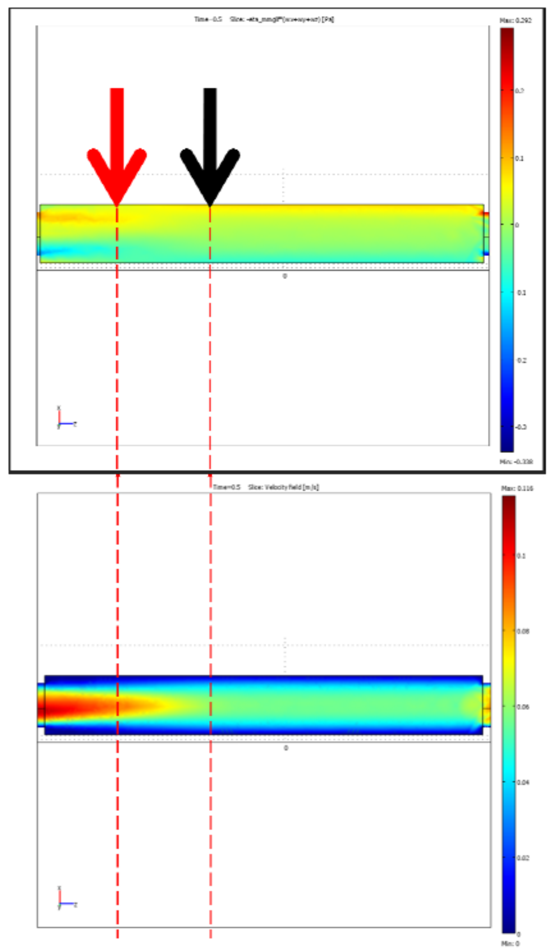

Figure 14.5 An illustration of how velocity profile relates to wall shear stress.

It can be seen that the smaller inlet diameter shields the proximal scaffold wall until the velocity profile fans out to the width of the scaffold. The scaffold is 3 cm long and approximately 1 cm of the length received a wall shear stress significantly less than the recorded max of 2 dynes/cm^2. The angled inlet and outlet have a negligible effect upon the shear stress as illustrated by the symmetrical and centered nature of the velocity profile. An example of a case where the angled inlet has an effect on velocity profile is shown in Figure 14.6.

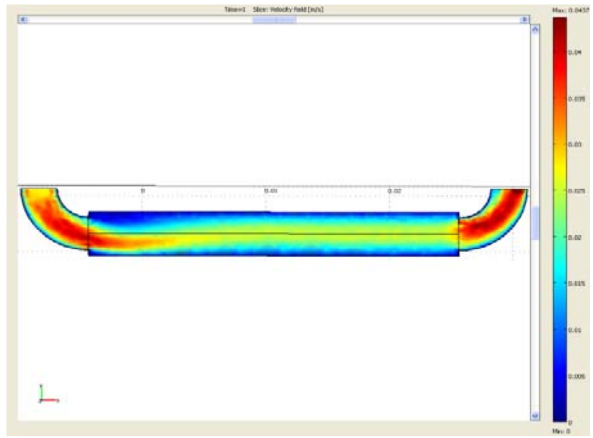

Figure 14.6 Longitudinal cross section of velocity profile with inlet bias.

Since the geometries leading to the scaffold in the design did not have a noticeable effect upon the velocity profile or corresponding wall shear stress, only the barb and scaffold interface would need to be resolved to allow for a uniform shear stress distribution. Extending the length of the scaffold is not a reasonable solution because ePTFE is expensive and it would be a waste of resources. A simple solution would be to use a larger diameter barb that would stretch the scaffold slightly and provide a more even transition from the fitting to the scaffold.

Limitations of this model stem from the governing equation used to estimate the inlet boundary conditions. From the Navier-Stokes equation, the relationship between the maximum and minimum velocities can be derived for a parabolic velocity profile for fully developed laminar pipe flow. The fluid dynamics and model do not meet these requirements since flow within the system is pulsatile and the pipe has varying geometries and diameters. The model is also limited in that the mesh density is low because the operating computer did not have enough memory to complete the simulation with higher densities. Importing the bioreactor assembly from SolidWorks into

COMSOL prevented further modifications to the geometry. As a result, the model could not be simplified and further meshed. Future work would include implementing a volumetric flowrate equation that more accurately accounted for the pulsatile nature of the flow conditions and recreating the bioreactor model within COMSOL. Creating the model within COMSOL would allow for geometry simplifications and greater mesh densities to be applied. It would also be useful to implement mixed boundary equations to further define environmental conditions.

The computational model can help predict the necessary viscosity to generate the ideal wall shear stresses. Dextran is used to alter the rheological properties of media within bioreactors to generate more physiologic conditions. The dextran increases fluid viscosity and as a result increases the wall shear stress within the system. Marc Dawson worked with increasing the viscosity of the bioreactor system to help generate ideal wall shear stresses (12). Dextran is made up of glucose and, accordingly, raised suspicions of its influence upon the endothelial cells in various concentrations. The tonicity of a solution can greatly affect the viability of a cell. The concern is that if the solute concentration becomes too high as a result of adding dextran to the media, a hyperosmotic solution would be created. This would result in the net movement of water out of the endothelial cells in contact with the media and kill the cells. Rouleau et al. studied the effect of dextran concentrations upon endothelial cells for this very reason. Dextran was found to increase endothelial cell viability, decrease their attachment to the surfaces of culture plates for concentrations up to 17.5% (45), and was able to generate viscosities near 20 cP. With the ability to produce such high viscosities and not negatively impact endothelial cell viability, increased dextran concentrations can be used within the bioreactor system. It is important to note that while viability was nearly unaffected by the 20% concentration of dextran, it did elicit a change in phenotypic expression.

The gathered data and the computational model are extremely useful for the Cal Poly BVM lab. With these results, the bioreactor can be modified so that wall shear stresses are uniformly distributed across the scaffold. Also, any design modifications or proposed changes in conditions can be assessed before they are actually implemented using the computational model. For example, geometries can be assessed for their impact on flow, or viscosity can be manipulated until the desired wall shear stress is achieved.

Overall, the work in this chapter verified that the system modifications made to back-pressure the bioreactor did not have a significant impact upon the flow conditions within the bioreactor chamber. The computational model did, however, indicate a potential issue with the fit of the luer lock barbs and the ePTFE scaffolding. The discrepancy between the inner diameters of the two parts generated a region of non-uniform wall shear stress. As a result, modifications can be made to minimize the transition between the fittings and the scaffold to alleviate the problem. In addition, the computational model can be used to guide future work.

Chapter 15: DISCUSSION AND CONCLUSION

In summary, the goal of this thesis was to improve physiologic pressure conditions within an *in vitro* blood vessel bioreactor system intended to assess intravascular devices. This goal was significant in that it could potentially contribute towards developing more complex tissue engineered blood vessels that include a tunica media. The newly designed bioreactor chamber can be rotated 360°. This will prevent cell sedimentation and justify the development of an automatic rotary system for the chambers.

There were 4 specific aims that comprised this project, and each will be summarized in turn. The first aim was to design a new bioreactor chamber that could withstand backpressures. This was accomplished by designing a more robust bioreactor chamber capable of administering elevated compressive forces to sealed edges, and by appropriate material selection of the silicone end plates that functioned as gaskets. The outcome was a bioreactor chamber that could withstand pressures 0-1000 mmHg.

The second aim was to establish a realistic base-line pressure to mimic systolic/diastolic conditions. This was accomplished by assessing various backpressure designs through a variety of trials. Results from this work led to the selection of the water column technique for back-pressuring the bioreactor system. Refinements in the design resulted in accurate and consistent pressure waves within the physiologic range.

The third aim was to characterize flow conditions through computational modeling. This was addressed in Chapter 14 and was accomplished by creating a simplified physical model of the bioreactor system in SolidWorks and importing the design into COMSOL. COMSOL was then used to set boundary conditions, flow parameters, and fluid properties in order to accurately simulate the operational flow conditions of the bioreactor. Results from this work indicated that the system modifications made to backpressure the bioreactor did not have a significant impact upon the flow conditions. However, the model did indicate that the wall shear stress within the bioreactor chamber may be non-uniform due to the interface between the luer lock fittings and ePTFE scaffolding.

The fourth aim was to propose a methodology to prevent cell sedimentation. This is addressed in Appendix D of this thesis with a suggested design of a rotary rack for the bioreactor chambers. The design includes a conceptual model generated in SolidWorks, specified parts necessary to accomplish task, and a proposed method of operation. The design is simple and scalable. The rack will allow the bioreactor chambers to be continually rotated automatically without laboratory assistant aid. This will free up laboratory assistants to perform other work and generate uniform cell dispersion across the bioreactor scaffold.

Overall, this work led to the development of a durable, reliable system that will provide the desired operating pressures and support further experimentation and optimization. This work is similar to some of the bioreactor systems researched for this project. For example, this system incorporated a vented reservoir. Chen et al. used vented reservoirs in order to allow media within the bioreactor system to exchange gases with the CO2 within the incubator (9). This allowed the system to maintain the correct media pH level. In the recently designed bioreactor system, the vented reservoirs allow the system to adapt to dynamic volumetric conditions. In another study, performed by Engbers-Buijtenhuijs et al., a bioreactor system using a peristaltic pump was back-pressured to 100 mmHg (14). The newly designed bioreactor is also back-pressured to 100 mmHg. The systems are different in how the backpressure was established. In Engbers-Buijtenhuijs' trial, the system reservoir was pressurized – likely by compressed gas and a pressure regulator. In the new bioreactor system, a height differential was used to backpressure the bioreactor chamber to 100 mmHg.

Although significant progress was made through the accomplishments described in this thesis, there were several limitations and areas for improvement that were noted throughout the project. These areas for future work will be addressed below.

Future Work

There are several modifications that could readily be made to the recently designed bioreactor system to improve functionality while maintaining the desired pressure performance. First, there is an issue of potential contamination with a vented reservoir. Even in an aseptic incubator environment, the bioreactor cannot be open to the environment while maintaining sterility. To address this, the vented reservoir could be fitted with a purifying 0.22 micron filter which could easily be attached to the third panel mount in the cap using additional silicone tubing. The filter should be more than adequate to compensate for any pressure/volume fluctuation while ensuring sterile conditions.

There is also another potential solution to the problem of contamination. The filtered system is sterile, but still open to the incubator environment. The current reservoir is a hard-walled 50 ml conical tube. The non-compliant container cannot adjust to changing pressures. If the reservoir was designed to be more compliant, it could change shape to adjust its volume and compensate for pressure fluctuations. IV style bags could be used to accomplish this, but would have to be configured so that the reservoir inlet returned to the airspace above the liquid level. If the inlet fed into the base of the bag, below the liquid level, fluid level heights within the bag would contribute to the base-line pressures and result in pressure variation. Another reservoir could also be designed to accomplish this task – if the IV bag or some variant is not practical. Another perk to using an IV bag would be the ability to suspend the reservoir from a rack and save space. These bags also have greater volume capacities than the current reservoirs and would not require the cap modifications or the expense of air purifying filters. The current reservoirs have sealant issues that do not withstand multiple sterilizations or uses. IV bags could be purchased already sterile.

The settings for the bioreactor reservoir height (54 inches) used in this work was based on water density at room temperature. Two main differences that must be considered during the use of the bioreactors to tissue engineer vessels are media density and environmental temperature. While a temperature/density chart for the DMEM media would be desirable for performing the necessary calculations to determine the appropriate reservoir heights, water is quite similar. Assuming that water and media have roughly the same density, a water temperature/density chart would be sufficient for providing necessary data to perform the needed calculations. A new height would be calculated based on 37 ˚C. Of course, if the mounting hardware for the reservoir was adjustable, the appropriate height for a given fluid and temperature can be arrived at empirically using feedback from the pressure transducer data acquisition equipment placed within the incubator. Additional detailed recommendations for future work regarding modifying the bioreactor chamber, back-pressuring the system, and designing a rotary bioreactor chamber rack can be found in Appendix D.

Another topic of interest for future research would be developing a more physiologic pressure waveform within the bioreactor. All of the experiments and designs conducted for this thesis were performed under the generalized definition of physiologic conditions for arterial pressure – the range of pressures and frequency of the wave. The coronary artery has a characteristic pressure wave that resembles a skewed parabola where the pressure steadily increases to a maximum and then, at a slightly slower rate, decreases back to a minimum (Figure 15.1). The pressure waves generated within the bioreactor system do not resemble the characteristic coronary artery waves very closely with regard to general shape, rates of increase and decrease, and the general smoothness of the curve. Now that constant backpressure has been achieved, efforts can be made to create a more accurate wave. Potential areas of investigation would include pumps, dampeners, and system geometry variations.

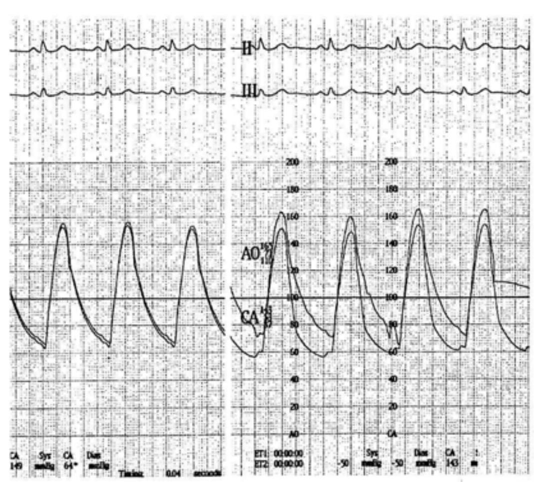

Figure 15.1 Characteristic coronary artery pressure waveform (23).

Conclusion

In summary, the ultimate goal of this thesis was to develop a method for providing accurate and sustainable pressure waves that are easily set and would replicate human physiologic pressure conditions. The study began with characterizing the current system used in the BVM laboratory. A number of system flaws made consistent pressurization of the system in the desired operating range difficult. Identifying specific concerns and resolving them led to designing a new bioreactor chamber. The chamber was redesigned and the bioreactor system modified to support physiologic pressure. Additionally, this robust and relatively user-friendly system provides a platform for future designs and optimization of the system.

After the manual pressure injection technique failed, pressure leak diagnostics confirmed the futility of patching up all of the micro leaks within the system. New methods intended to withstand system pressure-loss tendencies were employed. They ranged from simple regulators to more elaborate weighted syringe systems. Each technique was tried and assessed for potential viability. The numerous failed experiments helped increase overall knowledge and understanding of the system and allowed a practical solution to be devised.

The water column technique proved quite effective in producing consistently accurate pressure waveforms. The pressures are easily adjusted and can mimic virtually any potentially desired pressure range. It is also worth noting that the system can be applied to more than just the pulsatile bioreactor. The continuous flow perfusion system would react similarly to the back-pressuring, but there would not be discernable pulses.

Some technical issues were encountered with the data acquisition equipment, specifically the transducer zero calibration. The experiments were well documented – materials, set up, operation, observations and results were characterized in meticulous detail. The completeness of records allowed questionable or confusing results to be reviewed both during and after experimentation. Some of the results indicated system generated phenomena, such as vacuum formation, that needed to be dealt with. Other results indicated pressurization of an atmospherically vented open system – which is impossible. This led to trouble-shooting analysis and deconstruction of the system in search of evidence for or against the hypothesized explanation. As it turned out, the pressure transducer demonstrated random drift with no causal correlation. Further tests were required to confirm the impression that the system was still and had been operating at the appropriate pressures even though the transducer indicated contrary measurements.

Computational modeling performed using COMSOL provided valuable data characterizing the flow conditions within the newly designed bioreactor system. Using a height differential to establish the backpressure within the bioreactor chamber did not significantly influence the velocity profile of the fluid flowing through the scaffold. As a result, the wall shear stress distribution was not affected by that system modification. The COMSOL model also provided important information regarding the effect of the luer lock fittings and scaffold interface which resulted in a non-uniform wall shear stress distribution. The computational model will help future work when modifying system designs and flow conditions to improve the distribution and magnitude of wall shear stress.

Ultimately and overall, the system proved to be a success. There is future work to be done assembling a bioreactor chamber rack and designing more scalable back-pressuring systems, but based on the work performed in this project, the lab has a durable, reliable system that will provide the desired operating pressures and support further experimentation and optimization. The blood vessel mimic can now run under the human condition of 120/80mmHg. In addition, significant progress was made in characterizing and documenting the intricacies of the pressure/flow environment. The research and progress made during this project will help direct future work and improvements to the bioreactor system necessary to create an effective assay for intravascular devices.

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APPENDIX A: Raw Data

Contains:

1) Table A.1: 25 hour test

2) Table A.2: 31 hour test

3) Table A.3: 18 hour test

4) Table A.4: 72 hour test

Data is recorded at a minutely rate for all of the above trials

5) Table A.5: Weighted Syringe

Table A.1: 25 Hour Test

Minimum	maximum	height
mmHg	mmHg	mmHg
79.7915	119.6055	39.814
85.2281	123.9829	38.7548
84.4436	123.4373	38.9938
83.8755	122.9706	39.0951
83.4919	122.6164	39.1245
83.2121	122.3973	39.1852
82.9851	122.1949	39.2098
82.7523	122.0734	39.3211
82.5982	121.9574	39.3592
82.457	121.882	39.425
82.4458	121.7833	39.3376
82.4579	121.6404	39.1826
82.3315	121.5859	39.2544
82.2102	121.5218	39.3116
82.0796	121.4453	39.3657
82.0171	121.4014	39.3843
81.9582	121.3529	39.3947
81.8742	121.3114	39.4371
81.8327	121.2464	39.4138
81.7521	121.1858	39.4337
81.6716	121.1693	39.4978
81.6274	121.1642	39.5367
81.6832	121.0832	39.4
81.6889	121.0412	39.3523
81.6473	120.997	39.3497
81.5893	120.9399	39.3505
81.5426	120.9156	39.3731
81.4724	120.8619	39.3895
81.3815	120.8082	39.4268
81.3183	120.8221	39.5038
81.2715	120.7918	39.5203
81.1934	120.7916	39.5982
81.1148	120.7485	39.6337
81.0212	120.7156	39.6943
80.9468	120.6619	39.7151
80.9165	120.6489	39.7324
80.8896	120.616	39.7264
80.8342	120.5684	39.7342
80.7563	120.5311	39.7749
80.6657	120.5238	39.8581
80.6168	120.4826	39.8658
80.5562	120.4714	39.9152
80.5614	120.4411	39.8797
80.6342	120.3562	39.7221
80.6255	120.3077	39.6822
80.5623	120.2748	39.7125
80.4921	120.267	39.7749
80.4922	120.2287	39.7365
80.3899	120.157	39.7671
80.3605	120.1224	39.7619
80.312	120.1146	39.8026
80.2419	120.086	39.8442
80.2107	120.08	39.8693
80.1864	120.0445	39.858
80.125	120.0038	39.8788
80.0694	119.9583	39.8889
79.989	119.9137	39.9247
79.9007	119.8808	39.9801
79.8886	119.8384	39.9498
79.8764	119.821	39.9446
79.8357	119.8219	39.9862
79.7959	119.7985	40.0026
79.7301	119.7803	40.0503
79.6615	119.7434	40.0819
79.5993	119.7292	40.1299
79.5863	119.6712	40.0849
79.5145	119.6366	40.1221
79.4893	119.608	40.1187
79.5179	119.5708	40.0529
79.6625	119.4902	39.8277
79.6114	119.4565	39.845
79.5954	119.421	39.8255
79.5621	119.3984	39.8364
79.5049	119.3811	39.8762
79.446	119.3378	39.8918
79.44	119.3231	39.8831
79.4105	119.2668	39.8563
79.33	119.2374	39.9074
79.3006	119.2426	39.942
79.3023	119.2365	39.9342
79.2801	119.2386	39.9585
79.2538	119.2192	39.9654
79.2451	119.1932	39.9481
79.2928	119.1109	39.8182
79.2053	119.0581	39.8528
79.136	119.0685	39.9325
79.1352	119.0062	39.871
79.1308	118.9862	39.8554
79.0687	118.9638	39.8951
78.9914	118.9499	39.9585
78.9568	118.9455	39.9888
78.9256	118.923	39.9974
78.8936	118.9256	40.0321
78.8702	118.904	40.0338
78.8589	118.8598	40.0009
78.8182	118.8434	40.0251
78.7727	118.8175	40.0449
78.7507	118.7628	40.0122
78.7117	118.7671	40.0554
78.6996	118.7005	40.0009
78.6571	118.6849	40.0277
78.5974	118.6693	40.0719
78.5454	118.6676	40.1221
78.5385	118.6217	40.0832
78.5058	118.6096	40.1039
78.4874	118.5957	40.1083
78.4701	118.5515	40.0814
78.4034	118.4918	40.0884
78.4034	118.4684	40.065
78.3523	118.4372	40.0849
78.3004	118.4112	40.1109
78.2233	118.4173	40.194
78.149	118.3753	40.2263
78.1124	118.3472	40.2347
78.1306	118.342	40.2113
78.1696	118.29	40.1204
78.1306	118.2658	40.1351
78.0492	118.2476	40.1983
77.9756	118.2536	40.278
77.9289	118.2372	40.3083
77.9482	118.1771	40.229
77.9938	118.1497	40.1559
77.986	118.1255	40.1394
77.9583	118.1081	40.1498
77.9609	118.0285	40.0676
77.883	117.993	40.11
77.7998	117.9912	40.1914
77.8197	117.9549	40.1351
77.7929	117.9159	40.123
77.7376	117.9287	40.1911
77.7479	117.8657	40.1178
77.6725	117.838	40.1654
77.6301	117.831	40.2009
77.6526	117.7332	40.0806
77.6223	117.7401	40.1178
77.631	117.7245	40.0936
77.5686	117.7072	40.1386
77.4769	117.7464	40.2695

77.3798	117.7237	40.3438	76.2506	116.675	40.4244	75.6358	115.7657	40.1299
77.3417	117.6856	40.3438	76.2181	116.6135	40.3955	75.7527	115.7588	40.0061
77.3634	117.663	40.2997	76.1874	116.5832	40.3958	75.647	115.7371	40.0901
77.3287	117.6518	40.323	76.1831	116.5832	40.4001	75.5741	115.7485	40.1744
77.4318	117.5505	40.1187	76.0904	116.5953	40.5049	75.4877	115.7146	40.2269
77.4309	117.5323	40.1013	76.1216	116.5477	40.4261	75.4565	115.725	40.2685
77.3824	117.5262	40.1438	76.1424	116.5347	40.3923	75.4366	115.6964	40.2598
77.3738	117.4557	40.0819	76.1588	116.5174	40.3585	75.2963	115.7025	40.4062
77.3694	117.405	40.0355	76.1597	116.4377	40.278	75.3396	115.6549	40.3152
77.3487	117.3851	40.0364	76.1749	116.4259	40.251	75.3465	115.6315	40.2849
77.3504	117.3816	40.0312	76.1606	116.3788	40.2183	75.2989	115.6245	40.3256
77.3885	117.3296	39.9411	76.1484	116.3537	40.2053	75.3469	115.597	40.2501
77.4457	117.2985	39.8528	76.1865	116.3416	40.155	75.3682	115.5657	40.1975
77.3565	117.2751	39.9186	76.2125	116.3121	40.0996	75.3985	115.5535	40.155
77.3106	117.25	39.9394	76.2047	116.2844	40.0797	75.3786	115.5076	40.1291
77.2619	117.2601	39.9982	76.1926	116.268	40.0754	75.3751	115.492	40.1169
77.2222	117.2249	40.0026	76.145	116.2783	40.1334	75.4141	115.4808	40.0667
77.1971	117.2205	40.0234	76.0806	116.2444	40.1638	75.4046	115.4418	40.0373
77.1755	117.2205	40.0451	76.0263	116.2169	40.1905	75.3725	115.4695	40.097
77.1192	117.2197	40.1005	75.9977	116.2402	40.2425	75.3389	115.4543	40.1153
77.1114	117.1833	40.0719	76.009	116.2125	40.2035	75.3361	115.4834	40.1472
77.0767	117.1781	40.1013	75.9397	116.164	40.2243	75.4124	115.4106	39.9983
77.0153	117.2049	40.1897	75.8826	116.158	40.2754	75.4331	115.3899	39.9567
76.9422	117.2002	40.258	75.8601	116.1606	40.3005	75.4297	115.3968	39.9671
76.8386	117.1893	40.3507	75.8973	116.1822	40.2849	75.4184	115.3916	39.9732
76.8031	117.1504	40.3473	75.8948	116.1484	40.2536	75.4011	115.3682	39.9671
76.8048	117.1201	40.3152	75.8497	116.0982	40.2486	75.4323	115.3232	39.8909
76.8222	117.088	40.2659	75.9709	116.0038	40.0329	75.4411	115.3301	39.8889
76.8533	117.0343	40.181	75.9328	116.0004	40.0676	75.4998	115.2859	39.7861
76.8525	117.0118	40.1594	75.8973	116.0333	40.136	75.4825	115.2435	39.761
76.8603	116.9685	40.1083	75.8531	116.0324	40.1793	75.4245	115.2245	39.8
76.8259	116.9174	40.0916	75.783	115.9865	40.2035	75.3708	115.1829	39.8121
76.7962	116.8828	40.0866	75.7198	115.9839	40.2641	75.2747	115.1985	39.9238
76.7096	116.8967	40.1871	75.745	115.959	40.214	75.227	115.2063	39.9792
76.6749	116.9088	40.2338	75.6886	115.9597	40.2711	75.1898	115.2028	40.013
76.6342	116.8958	40.2616	75.8176	115.9302	40.1126	75.1865	115.1962	40.0096
76.6256	116.8715	40.246	75.8531	115.912	40.0589	75.1448	115.2019	40.0572
76.5805	116.849	40.2685	75.8332	115.9224	40.0892	75.1967	115.2019	40.0052
76.5572	116.83	40.2728	75.7795	115.9068	40.1273	75.2218	115.1881	39.9663
76.4914	116.8023	40.3109	75.8133	115.9103	40.097	75.2348	115.1855	39.9507
76.4489	116.7923	40.3435	75.8081	115.8887	40.0806	75.1967	115.1569	39.9602
76.4281	116.7564	40.3282	75.8895	115.8692	39.9797	75.1387	115.169	40.0303
76.3883	116.7538	40.3655	75.9163	115.8471	39.9307	75.2106	115.1128	39.9022
76.2801	116.752	40.472	75.8938	115.828	39.9342	75.1883	115.1274	39.9392
76.2307	116.746	40.5153	75.815	115.7934	39.9784	75.1119	115.0946	39.9827
76.2082	116.7512	40.543	75.7336	115.7917	40.058	75.0755	115.098	40.0225
76.1727	116.7261	40.5534	75.6548	115.7562	40.1013	75.0937	115.0703	39.9766

75.085	115.0946	40.0096	74.2753	114.5048	40.2295	73.0539	113.3427	40.2889
75.053	115.0617	40.0087	74.3437	114.4875	40.1438	73.0933	113.2985	40.2053
75.0538	115.066	40.0121	74.3905	114.4061	40.0156	73.0197	113.2552	40.2356
75.0443	115.027	39.9827	74.3602	114.3905	40.0303	72.9158	113.283	40.3672
75.0634	115.0539	39.9905	74.329	114.4139	40.0849	72.8525	113.2847	40.4321
75.0914	115.0446	39.9532	74.2277	114.2312	40.0035	72.8413	113.2942	40.4529
75.1119	114.9837	39.8719	74.1437	114.1126	39.9689	72.9634	113.2656	40.3022
75.1162	114.9898	39.8736	74.1259	114.0704	39.9444	73.0058	113.2353	40.2295
75.1153	114.9681	39.8528	74.1134	114.058	39.9446	73.0257	113.2256	40.1999
75.0651	114.95	39.8849	74.0484	114.026	39.9775	73.0993	113.179	40.0797
75.0391	114.9387	39.8996	73.9541	114.0364	40.0823	73.0855	113.1669	40.0814
75.0036	114.9162	39.9126	73.8926	113.9896	40.097	73.0301	113.1617	40.1317
74.9101	114.911	40.0009	73.9497	113.9697	40.0199	73.0586	113.1098	40.0511
74.9187	114.8834	39.9647	73.935	113.9515	40.0165	73.0699	113.0907	40.0208
74.917	114.8893	39.9723	73.922	113.9437	40.0217	73.0283	113.0898	40.0615
74.9317	114.8893	39.9576	73.8934	113.9585	40.0651	73.0093	113.089	40.0797
74.9032	114.8919	39.9888	73.8908	113.922	40.0312	72.9703	113.0786	40.1083
74.9153	114.8867	39.9714	73.8813	113.8666	39.9853	73.0063	113.0829	40.0766
74.9439	114.8564	39.9126	73.8406	113.8528	40.0122	72.9686	113.05	40.0814
74.9162	114.8278	39.9117	73.7592	113.8606	40.1013	72.9738	113.0084	40.0347
74.8408	114.8287	39.9879	73.7488	113.8684	40.1195	72.9253	113.0119	40.0866
74.8289	114.8165	39.9876	73.6847	113.8268	40.142	73.0171	112.9851	39.968
74.7005	114.8149	40.1143	73.6631	113.8138	40.1507	73.0214	112.9851	39.9637
74.7075	114.8382	40.1308	73.6987	113.8008	40.1021	72.9868	113.0171	40.0303
74.6607	114.8045	40.1438	73.6614	113.7359	40.0745	72.9357	113.0275	40.0918
74.6676	114.7776	40.11	73.6631	113.7133	40.0503	72.9191	113.0159	40.0968
74.6875	114.7326	40.0451	73.6059	113.7367	40.1308	72.8803	112.9972	40.1169
74.7187	114.6893	39.9706	73.5427	113.7402	40.1975	72.8898	112.9868	40.097
74.73	114.678	39.9481	73.4613	113.7393	40.278	72.9573	112.9089	39.9515
74.7232	114.6958	39.9726	73.4786	113.7038	40.2252	72.992	112.8491	39.8571
74.6763	114.6841	40.0078	73.5332	113.6666	40.1334	72.9625	112.856	39.8935
74.6278	114.7335	40.1057	73.4961	113.6537	40.1576	72.921	112.8612	39.9403
74.5966	114.6633	40.0667	73.4639	113.6562	40.1923	72.8681	112.8136	39.9455
74.5905	114.7196	40.1291	73.4665	113.619	40.1524	72.8275	112.7922	39.9647
74.5074	114.7049	40.1975	73.4726	113.5402	40.0676	72.7737	112.7946	40.0208
74.4676	114.7032	40.2356	73.4475	113.5644	40.1169	72.7616	112.7937	40.0321
74.5325	114.6607	40.1282	73.3176	113.5583	40.2408	72.6906	112.8093	40.1187
74.6201	114.5795	39.9594	73.2838	113.554	40.2702	72.6612	112.8058	40.1446
74.5983	114.5629	39.9645	73.2128	113.5376	40.3248	72.6646	112.7859	40.1213
74.5117	114.5862	40.0745	73.2353	113.5004	40.2651	72.6612	112.7816	40.1204
74.4667	114.5724	40.1057	73.1634	113.4726	40.3092	72.7105	112.759	40.0485
74.4009	114.5386	40.1377	73.192	113.425	40.233	72.6848	112.7437	40.059
74.3792	114.5447	40.1654	73.1288	113.4224	40.2936	72.6672	112.7097	40.0425
74.2866	114.5594	40.2728	73.1426	113.4155	40.2728	72.5884	112.714	40.1256
74.2433	114.5248	40.2815	73.1357	113.3834	40.2477	72.6092	112.7054	40.0962
74.2915	114.487	40.1955	73.1115	113.3722	40.2607	72.5953	112.6742	40.0788
74.2736	114.5135	40.2399	73.0309	113.3964	40.3655	72.6187	112.6179	39.9992

72.6083	112.6049	39.9966	77.5184	117.586	40.0676	76.2064	116.5788	40.3724
72.5711	112.5936	40.0225	77.4465	117.5816	40.1351	76.1718	116.5234	40.3516
72.5077	112.5922	40.0845	77.4353	117.5634	40.1282	76.1657	116.5174	40.3516
72.4438	112.5478	40.1039	77.5201	117.4994	39.9792	76.138	116.4732	40.3352
72.3364	112.5148	40.1784	77.4883	117.4601	39.9717	76.1536	116.4749	40.3213
72.2992	112.501	40.2018	77.4379	117.4362	39.9983	76.2004	116.4368	40.2364
72.3165	112.4672	40.1507	77.366	117.418	40.052	76.1485	116.4321	40.2836
72.2334	112.456	40.2226	77.321	117.4041	40.0832	76.0627	116.4048	40.3421
72.2282	112.4248	40.1966	77.2075	117.4162	40.2087	75.9882	116.4308	40.4425
72.2576	112.4023	40.1446	77.1971	117.3972	40.2001	75.9596	116.4082	40.4486
72.2329	112.4028	40.17	77.1504	117.327	40.1767	75.9034	116.4056	40.5023
72.2178	112.3936	40.1758	77.1815	117.2526	40.071	75.8202	116.3979	40.5776
72.2091	112.346	40.1369	77.1368	117.2222	40.0854	75.7908	116.3615	40.5707
72.197	112.3434	40.1464	77.0932	117.1989	40.1057	75.8003	116.3286	40.5283
72.2516	112.346	40.0944	77.0369	117.1443	40.1074	75.7459	116.2946	40.5487
72.3399	112.3148	39.9749	77.0352	117.1374	40.1022	75.731	116.274	40.543
72.3217	112.3053	39.9836	76.972	117.1045	40.1325	75.6877	116.2584	40.5707
72.2992	112.2767	39.9775	76.9295	117.1054	40.1758	75.647	116.2316	40.5846
72.2975	112.2499	39.9524	76.9148	117.0612	40.1464	75.6886	116.2203	40.5317
72.2611	112.2399	39.9788	76.9191	117.0595	40.1403	75.6964	116.158	40.4616
72.1667	112.2265	40.0598	76.8507	117.0447	40.194	75.8003	116.106	40.3057
72.0636	112.2395	40.1758	76.8188	117.024	40.2052	75.8055	116.061	40.2555
71.9918	112.2299	40.2382	76.8594	117.0006	40.1412	75.7509	116.0627	40.3118
71.9961	112.1988	40.2027	76.8161	116.991	40.175	75.7177	116.0233	40.3056
72.0048	112.1953	40.1905	76.7823	116.9304	40.1481	75.705	115.9744	40.2693
71.9459	112.1832	40.2373	76.7468	116.9296	40.1827	75.6756	115.9683	40.2927
71.8489	112.1685	40.3196	76.7927	116.8759	40.0832	75.641	115.9571	40.3161
71.8303	112.1694	40.3391	76.739	116.8551	40.1161	75.6046	115.9181	40.3135
71.8359	112.1407	40.3048	76.6897	116.817	40.1273	75.5648	115.8869	40.3222
71.8671	112.1035	40.2364	76.6215	116.8153	40.1937	75.5847	115.8558	40.2711
71.8801	112.0767	40.1966	76.5901	116.8083	40.2183	75.5526	115.8705	40.3178
71.8688	112.0377	40.1689	76.6109	116.791	40.1802	75.6023	115.8137	40.2114
71.8749	111.9944	40.1195	76.6299	116.7581	40.1282	75.5829	115.7787	40.1957
71.816	112.0212	40.2053	76.5762	116.7512	40.175	75.5717	115.8159	40.2442
71.8212	111.9892	40.168	76.5901	116.7252	40.1351	75.5336	115.7873	40.2538
71.8153	111.9782	40.1629	76.377	116.7746	40.3975	75.4721	115.7666	40.2945
71.7614	111.9996	40.2382	76.293	116.8066	40.5135	75.4479	115.7683	40.3204
71.6844	112.0264	40.3421	76.3335	116.78	40.4466	75.4383	115.751	40.3126
71.667	112.0204	40.3533	76.3	116.7451	40.4451	75.3665	115.7328	40.3663
71.9138	112.2568	40.343	76.2948	116.7702	40.4754	75.3292	115.7291	40.3999
77.0473	117.1486	40.1013	76.2835	116.7269	40.4434	75.3613	115.7007	40.3395
77.6639	117.7392	40.0754	76.2757	116.7122	40.4365	75.3985	115.6652	40.2667
77.6414	117.741	40.0996	76.2411	116.7174	40.4763	75.4124	115.6609	40.2486
77.5817	117.7094	40.1277	76.2541	116.6897	40.4356	75.3682	115.6445	40.2763
77.579	117.728	40.149	76.2766	116.6897	40.4131	75.4098	115.6531	40.2434
77.5141	117.6786	40.1646	76.2947	116.6197	40.325	75.4894	115.5916	40.1022
77.4881	117.6526	40.1646	76.248	116.5875	40.3395	75.55	115.5215	39.9714

75.5362	115.5177	39.9814	74.9006	115.2236	40.323	74.6468	114.9707	40.3239
75.5336	115.512	39.9784	74.9716	115.2045	40.233	74.6624	114.9811	40.3187
75.5223	115.5016	39.9792	74.9628	115.212	40.2492	74.7049	114.982	40.2771
75.5145	115.5024	39.9879	74.9369	115.1864	40.2494	74.7611	114.9863	40.2252
75.4686	115.4678	39.9992	74.9213	115.1933	40.2719	74.7866	114.9821	40.1955
75.3665	115.492	40.1256	74.9049	115.2054	40.3005	74.7031	115.0019	40.2988
75.2851	115.4661	40.181	74.8971	115.1933	40.2962	74.6356	115.0218	40.3862
75.2288	115.4765	40.2477	74.8997	115.1907	40.291	74.5559	115.0556	40.4997
75.1698	115.486	40.3162	74.8512	115.1422	40.291	74.51	115.0547	40.5447
75.1681	115.473	40.3048	74.8867	115.1483	40.2616	74.5005	115.053	40.5525
75.2132	115.4349	40.2217	74.8932	115.1178	40.2246	74.5273	115.021	40.4936
75.2825	115.4063	40.1239	74.8772	115.1015	40.2243	74.5905	115.001	40.4105
75.2738	115.402	40.1282	74.8841	115.0937	40.2096	74.5849	114.9883	40.4034
75.2262	115.3578	40.1317	74.8893	115.0842	40.1949	74.581	114.995	40.414
75.1976	115.376	40.1784	74.8625	115.1006	40.2382	74.5879	115.0088	40.4209
75.098	115.3803	40.2823	74.8694	115.0634	40.194	74.5914	114.9768	40.3854
75.0791	115.3556	40.2765	74.8677	115.0669	40.1992	74.6183	114.9699	40.3516
75.0599	115.3639	40.304	74.9101	115.066	40.1559	74.6841	114.9032	40.2191
75.0712	115.3544	40.2832	74.9011	115.0332	40.1321	74.6832	114.9101	40.2269
75.0755	115.3318	40.2564	74.8919	115.0374	40.1455	74.7005	114.9049	40.2044
75.0443	115.3327	40.2884	74.8729	115.0322	40.1594	74.6624	114.8737	40.2114
75.0053	115.3578	40.3525	74.8218	115.0097	40.1879	74.6286	114.8833	40.2546
74.9664	115.3578	40.3914	74.7767	115.0288	40.252	74.6087	114.9101	40.3014
75.0209	115.3925	40.3715	74.71	115.0288	40.3187	74.6027	114.9352	40.3326
75.0331	115.3102	40.2771	74.6278	115.0513	40.4235	74.6027	114.943	40.3404
75.1178	115.2939	40.1761	74.5481	115.0513	40.5032	74.5879	114.9361	40.3481
75.1223	115.3544	40.2321	74.5377	115.0339	40.4962	74.581	114.9118	40.3308
75.1084	115.2981	40.1897	74.5734	115.0341	40.4606	74.5628	114.9482	40.3854
75.1352	115.3119	40.1767	74.5455	115.0573	40.5118	74.5602	114.9151	40.3549
75.1465	115.279	40.1325	74.5109	115.0513	40.5404	74.5507	114.8945	40.3438
75.0781	115.3145	40.2364	74.6174	114.9924	40.375	74.4918	114.9067	40.4148
75.0296	115.2937	40.2641	74.5836	114.9933	40.4096	74.4581	114.937	40.4789
75.0045	115.2712	40.2667	74.5498	114.9958	40.446	74.4598	114.8893	40.4295
75.0799	115.2543	40.1744	74.4996	114.9967	40.4971	75.2374	114.497	39.2596
75.1664	115.176	40.0096	74.4122	115.0383	40.6261	75.2842	114.3594	39.0752
75.1526	115.195	40.0425	74.3417	115.0411	40.6994	75.3916	114.2961	38.9046
75.1274	115.2071	40.0797	74.3879	115.0374	40.6495	75.3413	114.2658	38.9245
75.0686	115.2019	40.1334	74.3715	115.0781	40.7067	75.2975	114.2765	38.979
75.0504	115.2435	40.1931	74.4745	115.0513	40.5768	75.3128	114.2762	38.9635
75.0729	115.2141	40.1412	74.5031	115.0348	40.5317	75.2521	114.2502	38.9981
75.0616	115.1933	40.1317	74.497	115.0409	40.5439	75.2279	114.2901	39.0622
75.0491	115.1838	40.1347	74.5429	115.0262	40.4832	75.2262	114.2987	39.0726
74.9534	115.1976	40.2442	74.6001	114.9673	40.3672	75.2521	114.3126	39.0605
74.885	115.2816	40.3966	74.5998	114.9821	40.3822	75.2106	114.323	39.1124
74.8832	115.2989	40.4157	74.5706	114.9638	40.3932	75.1647	114.3013	39.1367
74.8434	115.2712	40.4278	74.5983	114.9612	40.3629	75.1997	114.2624	39.0627
74.8798	115.247	40.3672	74.6347	114.9491	40.3144	75.1647	114.2355	39.0708

75.1647	114.2425	39.0778	75.6948	113.3991	37.7043	75.3266	113.2622	37.9356
75.1933	114.1784	38.9851	75.673	113.3895	37.7165	75.3119	113.3167	38.0048
75.1352	114.1966	39.0613	75.6886	113.3895	37.7009	75.2931	113.2714	37.9783
75.0703	114.2303	39.16	75.6332	113.3704	37.7372	75.5431	113.1245	37.5814
75.0686	114.2303	39.1618	75.634	113.3522	37.7182	75.5405	113.0855	37.545
75.0954	114.2043	39.1089	75.6098	113.3678	37.758	75.5024	113.102	37.5996
75.0817	114.1822	39.1005	75.5674	113.3141	37.7468	75.4219	113.1297	37.7078
75.072	114.1559	39.0838	75.5613	113.3514	37.7901	75.4149	113.1513	37.7364
75.0859	114.174	39.0882	75.5054	113.3101	37.8047	75.4141	113.1158	37.7017
75.0677	114.1576	39.0899	75.5518	113.2778	37.726	75.4349	113.0656	37.6307
75.1049	114.1628	39.0578	75.5059	113.257	37.7511	75.3469	113.0705	37.7237
75.0746	114.1688	39.0942	75.5041	113.2223	37.7182	75.46	113.0613	37.6013
75.0686	114.2	39.1315	75.5119	113.2042	37.6922	75.5232	113.0483	37.5251
75.0937	114.1957	39.102	75.5596	113.1877	37.6281	75.3587	113.0717	37.713
75.0808	114.1787	39.0979	75.5007	113.1617	37.661	75.4418	113.0102	37.5684
75.0677	114.1948	39.1271	75.4392	113.1756	37.7364	75.4886	112.9825	37.4939
75.0235	114.168	39.1444	75.4649	113.1463	37.6814	75.4825	113.0301	37.5476
75.0426	114.1654	39.1228	75.4375	113.1557	37.7182	75.4808	113.0232	37.5424
74.9984	114.1463	39.1479	75.4634	113.1557	37.6922	75.4966	113.0468	37.5502
74.9698	114.168	39.1981	75.5249	113.1435	37.6186	75.1586	112.9807	37.8221
74.9681	114.1524	39.1843	75.5215	113.1383	37.6169	74.5966	113.1886	38.592
74.9239	114.1394	39.2155	75.4799	113.1236	37.6437	74.3108	113.2379	38.9271
74.9046	114.1364	39.2318	75.4539	113.1712	37.7173	74.226	113.6813	39.4553
74.9898	114.1169	39.1271	75.4539	113.1998	37.7459	74.1887	113.8173	39.6285
74.9854	114.1255	39.1401	75.4843	113.178	37.6937	74.1853	113.7714	39.5861
75.0919	114.142	39.0501	75.4981	113.1834	37.6853	74.239	113.7566	39.5177
74.9958	114.1593	39.1635	75.4574	113.1929	37.7355	74.229	113.7717	39.5428
74.9984	114.1403	39.1419	75.4747	113.1929	37.7182	74.219	113.7861	39.567
75.8038	113.5921	37.7883	75.4877	113.1929	37.7052	74.2208	113.7844	39.5636
75.8116	113.5774	37.7658	75.4591	113.2024	37.7433	74.2164	113.7896	39.5731
75.7609	113.563	37.8021	75.473	113.2189	37.7459	74.1801	113.7956	39.6155
75.7613	113.5523	37.7909	75.4764	113.2128	37.7364	74.0866	113.8277	39.7411
75.7406	113.5566	37.8161	75.4455	113.1639	37.7184	74.0943	113.8103	39.716
75.6773	113.5445	37.8671	75.4574	113.1409	37.6836	74.129	113.8077	39.6788
75.7371	113.502	37.765	75.4046	113.179	37.7745	74.0916	113.7779	39.6863
75.712	113.5124	37.8005	75.4409	113.1842	37.7433	74.1169	113.7419	39.6251
75.7137	113.4665	37.7528	75.4271	113.1998	37.7728	74.2069	113.7445	39.5376
75.6522	113.47	37.8178	75.4262	113.1678	37.7416	74.1775	113.7939	39.6164
75.6271	113.4492	37.8221	75.4574	113.1712	37.7139	74.1801	113.8233	39.6432
75.6058	113.4432	37.8373	75.4531	113.1574	37.7043	74.1662	113.8233	39.6571
75.615	113.4267	37.8117	75.5708	113.1002	37.5294	74.1316	113.8242	39.6926
75.5942	113.4085	37.8143	75.6023	113.1172	37.5149	74.161	113.7991	39.6381
75.5881	113.399	37.8109	75.5518	113.1773	37.6255	74.1662	113.8077	39.6415
75.6115	113.4042	37.7927	75.4938	113.2449	37.7511	74.1629	113.8131	39.6502
75.6262	113.4328	37.8065	75.4331	113.2397	37.8065	74.129	113.8207	39.6917
75.654	113.3999	37.7459	75.3829	113.2873	37.9044	74.1602	113.8328	39.6727
75.6843	113.4016	37.7173	75.3708	113.2985	37.9278	74.2199	113.8181	39.5982

74.2113	113.8484	39.6372	74.4044	114.0952	39.6909	74.4298	114.1479	39.718
74.1809	113.864	39.6831	74.4693	114.1022	39.6329	74.4191	114.1351	39.716
74.2104	113.8874	39.677	74.4771	114.103	39.6259	74.3767	114.1437	39.7671
74.1636	113.8909	39.7272	74.4788	114.1307	39.6519	74.3593	114.1533	39.7939
74.1497	113.9021	39.7524	74.4806	114.1255	39.645	74.3489	114.1498	39.8009
74.1723	113.9186	39.7463	74.4321	114.1169	39.6848	74.381	114.1611	39.7801
74.1809	113.9134	39.7324	74.3708	114.1153	39.7445	74.4329	114.1325	39.6995
74.161	113.9272	39.7662	74.3507	114.1152	39.7645	74.5074	114.0874	39.58
74.1047	113.9679	39.8632	74.3628	114.1316	39.7688	74.5452	114.1118	39.5665
74.0874	113.9818	39.8944	74.413	114.0935	39.6805	74.5455	114.1074	39.5618
74.0952	113.9428	39.8476	74.4122	114.071	39.6588	74.5498	114.1013	39.5515
74.0891	113.9394	39.8502	74.491	114.0294	39.5385	74.536	114.1411	39.6051
74.0942	113.9382	39.844	74.5308	114.0641	39.5333	74.5975	114.0944	39.4969
74.0805	113.9601	39.8797	74.549	114.0701	39.5211	74.4771	114.1022	39.6251
74.1827	113.9567	39.774	74.5082	114.0492	39.541	74.4122	114.0935	39.6813
74.1498	113.9532	39.8035	74.497	114.026	39.5289	74.4338	114.1463	39.7125
74.1273	113.9584	39.8312	74.497	114.0034	39.5064	74.4792	114.1311	39.652
74.1195	113.9939	39.8745	74.4563	114.0138	39.5575	74.5204	114.1472	39.6268
74.1628	113.9264	39.7636	74.4416	114.0312	39.5896	74.5299	114.1706	39.6406
74.2113	113.9134	39.7021	74.3922	114.026	39.6337	74.5031	114.2069	39.7039
74.2545	113.91	39.6555	74.3472	114.0563	39.7091	74.4936	114.2338	39.7402
74.2632	113.9177	39.6545	74.3169	114.1082	39.7913	74.5481	114.1966	39.6484
74.284	113.9238	39.6398	74.3259	114.0906	39.7647	74.5914	114.1706	39.5792
74.2338	113.9498	39.716	74.5117	114.0597	39.548	74.594	114.181	39.587
74.2831	113.9463	39.6632	74.4823	114.0554	39.5731	74.5849	114.2034	39.6185
74.258	113.9402	39.6822	74.4624	114.0658	39.6034	74.5983	114.1966	39.5982
74.2346	113.9974	39.7628	74.4511	114.084	39.6329	74.5888	114.2009	39.6121
74.2771	114.032	39.755	74.4442	114.0935	39.6493	74.555	114.2087	39.6536
74.281	114.0501	39.7691	74.4251	114.1273	39.7021	74.6113	114.1628	39.5515
74.3074	114.0597	39.7524	74.426	114.116	39.69	74.6382	114.1775	39.5393
74.2831	114.0623	39.7792	74.5232	114.1338	39.6106	74.6364	114.1749	39.5385
74.2597	114.0736	39.8138	74.4624	114.1437	39.6813	74.6087	114.1645	39.5558
74.2597	114.0597	39.8	74.4165	114.1559	39.7394	74.6915	114.1179	39.4265
74.3533	114.0779	39.7246	74.2953	114.0996	39.8043	74.7421	114.0459	39.3038
74.4096	114.0277	39.6181	74.2753	114.1082	39.8329	74.6962	114.0485	39.3523
74.4364	113.9939	39.5575	74.2459	114.0987	39.8528	74.6737	114.0303	39.3566
74.4492	114.0052	39.556	74.2684	114.116	39.8476	74.5637	114.0138	39.4501
74.4736	114.0147	39.5411	74.3074	114.1325	39.8251	74.5065	114.0199	39.5134
74.4529	114.013	39.5601	74.3779	114.1223	39.7445	74.4511	114.0519	39.6008
74.4624	114.0069	39.5445	74.4589	114.103	39.6441	74.3689	114.0667	39.6978
74.4953	114.0034	39.5082	74.3922	114.0926	39.7004	74.3197	114.0818	39.7621
74.5005	114.0398	39.5393	74.3342	114.0926	39.7584	74.3299	114.1074	39.7775
74.4381	114.0346	39.5965	74.3611	114.0961	39.735	74.3619	114.0693	39.7073
74.426	114.0641	39.6381	74.2987	114.0874	39.7887	74.3992	114.0389	39.6398
74.4321	114.1022	39.6701	74.2753	114.1143	39.839	74.5091	114.0502	39.5411
74.392	114.0836	39.6916	74.2978	114.1186	39.8208	74.5039	114.0545	39.5506
74.4139	114.0779	39.664	74.407	114.1524	39.7454	74.491	114.0597	39.5688

74.4875	114.0745	39.587	74.6148	113.3964	38.7816	73.9653	113.302	39.3367
74.5481	114.039	39.4908	74.5923	113.3938	38.8015	74.019	113.2933	39.2743
74.5849	114.0378	39.4529	74.562	113.3687	38.8067	73.9567	113.2856	39.3289
74.6096	114.0251	39.4155	74.5611	113.3938	38.8327	73.8649	113.302	39.4371
74.6797	114.0173	39.3376	74.562	113.4273	38.8653	73.8467	113.2639	39.4172
74.6988	114.0052	39.3064	74.5871	113.4354	38.8483	73.785	113.2687	39.4837
74.6607	114	39.3393	74.5758	113.4189	38.8431	73.7791	113.263	39.4839
74.5992	114.0182	39.419	74.5524	113.3782	38.8258	73.7254	113.263	39.5376
74.5905	113.9931	39.4025	74.5161	113.3661	38.85	73.7532	113.2734	39.5203
74.5438	113.9783	39.4345	74.4901	113.3929	38.9028	73.7384	113.2856	39.5471
74.5056	114.0351	39.5295	74.4563	113.3808	38.9245	73.7298	113.2717	39.5419
74.536	114.0173	39.4813	74.4407	113.3375	38.8968	73.7445	113.2249	39.4804
74.478	114.0667	39.5887	74.4237	113.3885	38.9649	73.7125	113.2284	39.516
74.4736	114.0537	39.58	74.381	113.3756	38.9946	73.7376	113.2284	39.4908
74.4866	114.0623	39.5757	74.3576	113.3773	39.0197	73.7242	113.23	39.5058
74.5065	114.0866	39.58	74.3437	113.3211	38.9773	73.7272	113.2293	39.5021
74.5014	114.0606	39.5592	74.3429	113.3297	38.9868	73.7073	113.2691	39.5618
75.0893	113.5427	38.4534	74.3039	113.3211	39.0171	73.9445	113.2552	39.3107
75.0271	113.3339	38.3068	74.2649	113.3011	39.0362	73.9004	113.2535	39.3531
75.0937	113.257	38.1633	74.2831	113.2795	38.9964	73.7696	113.2622	39.4926
75.2556	113.58	38.3244	74.281	113.2705	38.9895	73.7973	113.2665	39.4692
75.3197	113.6458	38.3261	74.2225	113.2856	39.063	73.8181	113.1816	39.3635
75.2703	113.6164	38.346	74.2563	113.2604	39.0042	73.7727	113.1868	39.4141
75.1985	113.6172	38.4188	74.2303	113.244	39.0137	73.7861	113.2189	39.4328
75.137	113.6285	38.4915	74.161	113.2578	39.0968	73.8259	113.2215	39.3956
75.0919	113.5878	38.4958	74.2831	113.2431	38.96	73.7601	113.263	39.503
75.1231	113.5744	38.4513	74.3611	113.289	38.928	73.9212	113.2518	39.3306
75.1049	113.6345	38.5296	74.2234	113.276	39.0527	73.7679	113.276	39.5082
75.1326	113.5722	38.4395	74.2545	113.2784	39.0239	73.7047	113.2864	39.5818
75.1292	113.5376	38.4084	74.1654	113.3011	39.1358	73.7644	113.2959	39.5315
75.0807	113.5774	38.4967	74.1307	113.3037	39.173	73.8696	113.2855	39.4159
75.0131	113.5921	38.579	74.0952	113.3011	39.2059	73.8423	113.3211	39.4787
74.9759	113.5938	38.6179	74.0043	113.2985	39.2943	73.8666	113.2985	39.432
74.9924	113.5878	38.5954	73.9662	113.3029	39.3367	73.7055	113.2778	39.5722
74.9654	113.5515	38.5861	74.0528	113.2856	39.2328	73.7419	113.2622	39.5203
74.9404	113.5731	38.6327	74.1506	113.3211	39.1704	73.722	113.2847	39.5627
74.9213	113.5912	38.6699	74.1224	113.2855	39.1631	73.8207	113.2873	39.4666
74.9118	113.5514	38.6396	73.9722	113.3098	39.3376	73.8294	113.2942	39.4649
74.9058	113.5843	38.6786	74.0926	113.2856	39.1929	73.9154	113.3101	39.3948
74.9101	113.606	38.6959	73.9359	113.3072	39.3713	73.9601	113.3176	39.3575
74.8806	113.5488	38.6682	73.9489	113.2882	39.3393	73.9359	113.3159	39.38
74.8434	113.5661	38.7227	73.9939	113.2925	39.2986	73.8952	113.315	39.4198
74.8588	113.5286	38.6698	74.0147	113.2994	39.2847	73.9108	113.3124	39.4016
74.7663	113.4536	38.6872	74.1039	113.2933	39.1895	73.7237	113.2925	39.5688
74.6823	113.4665	38.7842	73.8925	113.2767	39.3842	73.8068	113.2968	39.49
74.7075	113.4458	38.7383	73.8761	113.2994	39.4233	73.8675	113.2691	39.4016
74.6659	113.4163	38.7504	73.8934	113.2821	39.3887	73.7524	113.2423	39.4899

73.7913	113.2232	39.432	78.9152	117.6907	38.7755	77.5851	116.8395	39.2544
73.799	113.2345	39.4354	78.6899	117.7261	39.0362	77.5842	116.8352	39.251
73.7653	113.2353	39.4701	78.6805	117.6873	39.0068	77.6405	116.8317	39.1912
73.709	113.2908	39.5818	78.6528	117.6379	38.9851	77.6002	116.7994	39.1992
73.6769	113.2743	39.5974	78.7437	117.6345	38.8907	77.5773	116.7746	39.1973
73.7133	113.27	39.5567	78.5645	117.6431	39.0786	77.5478	116.7416	39.1938
73.6752	113.2604	39.5852	78.4277	117.6206	39.1929	77.5478	116.7425	39.1947
73.674	113.2546	39.5806	78.4329	117.6474	39.2146	77.5019	116.7304	39.2284
73.735	113.2388	39.5038	78.5446	117.5375	38.9929	77.4292	116.7425	39.3133
73.78	113.108	39.328	78.6317	117.5138	38.8821	77.4006	116.6854	39.2847
74.284	112.8959	38.6119	78.5498	117.4933	38.9435	77.3816	116.6845	39.3029
74.2164	112.9998	38.7833	78.4103	117.5193	39.1089	77.4196	116.7677	39.3481
74.2182	112.9885	38.7703	78.3151	117.521	39.2059	77.4049	116.7321	39.3272
74.2918	112.9669	38.6751	78.2692	117.4734	39.2042	77.3539	116.7122	39.3583
74.2234	112.9244	38.7011	78.2129	117.4621	39.2492	77.5773	116.4533	38.876
74.1731	112.9548	38.7816	78.1964	117.4197	39.2233	77.7565	116.1095	38.353
74.1294	112.9393	38.8098	78.2198	117.3799	39.16	77.5859	116.19	38.6041
73.8666	112.8439	38.9773	78.1861	117.3651	39.1791	77.3539	116.2125	38.8587
73.9298	112.8249	38.895	78.1199	117.3711	39.2512	77.5383	116.4316	38.8933
73.8787	112.7937	38.915	78.0873	117.3504	39.2631	77.4552	116.403	38.9479
73.7878	112.792	39.0042	78.0354	117.3452	39.3098	77.3659	116.4585	39.0926
73.5713	112.7963	39.225	78.0077	117.3037	39.296	77.3539	116.4541	39.1003
73.5843	112.7902	39.2059	78.0527	117.2993	39.2466	77.2967	116.429	39.1323
73.6025	112.7608	39.1583	78.0752	117.2872	39.212	77.3253	116.3849	39.0596
73.6071	112.7481	39.1411	78.0362	117.263	39.2267	77.2681	116.3857	39.1176
73.6042	112.7045	39.1003	78.0691	117.2404	39.1713	77.2525	116.3632	39.1107
73.6207	112.7209	39.1003	78.1252	117.2055	39.0803	77.2205	116.3355	39.115
73.541	112.7114	39.1704	78.0276	117.1599	39.1323	77.3651	116.2472	38.8821
73.5488	112.6846	39.1358	78.031	117.1703	39.1393	77.3157	116.2347	38.9191
75.8272	114.8201	38.9929	78.0155	117.1054	39.0899	77.2344	116.2273	38.9929
79.2018	118.1229	38.921	77.9384	117.1209	39.1826	77.159	116.2247	39.0656
79.1378	118.1523	39.0146	77.928	117.1166	39.1886	77.0975	116.2082	39.1107
79.1224	118.1436	39.0213	77.8795	117.1036	39.2241	77.0811	116.2013	39.1202
79.1759	118.0744	38.8985	77.8267	117.1149	39.2882	77.1313	116.1666	39.0353
79.0797	118.0744	38.9946	77.8442	117.1244	39.2802	77.217	116.1337	38.9167
79.0278	118.0354	39.0076	77.8475	117.075	39.2276	77.2361	116.087	38.8509
78.9983	118.0129	39.0146	77.8076	117.017	39.2094	77.1606	116.1088	38.9481
78.9801	117.9817	39.0016	77.8215	116.9434	39.1219	77.0334	116.1381	39.1046
78.9438	117.9479	39.0042	77.7271	116.9425	39.2155	77.0109	116.1398	39.1289
78.9741	117.9228	38.9487	77.7184	116.9244	39.2059	77.0334	116.1363	39.1029
78.9603	117.8864	38.9261	77.7002	116.9122	39.212	77.017	116.1476	39.1306
78.9776	117.8432	38.8656	77.7522	116.901	39.1488	76.9876	116.1129	39.1254
79.0364	117.7877	38.7513	77.735	116.9086	39.1737	76.881	116.1207	39.2397
78.9758	117.7947	38.8188	77.7288	116.9062	39.1774	76.9278	116.0541	39.1263
78.9039	117.7834	38.8795	77.6327	116.9036	39.2709	76.9052	116.0524	39.1472
78.8814	117.7609	38.8795	77.6483	116.8759	39.2276	76.8265	116.0419	39.2155
78.8693	117.7176	38.8483	77.5998	116.8837	39.2839	76.8005	116.035	39.2345

76.8092	116.009	39.1999	75.5691	115.1439	39.5748	74.6183	114.4789	39.8606
76.9572	115.9683	39.0111	75.6124	115.1647	39.5523	74.6324	114.4342	39.8017
76.7537	115.9813	39.2276	75.5613	115.1483	39.587	74.6191	114.381	39.7619
76.6871	115.9398	39.2527	75.5613	115.1569	39.5956	74.6745	114.3983	39.7238
76.7269	115.9207	39.1938	75.5483	115.0876	39.5393	74.6815	114.4105	39.729
76.7193	115.8903	39.171	75.7354	115.1015	39.3661	74.6875	114.3914	39.7039
76.7347	115.8826	39.1479	75.6349	115.0876	39.4527	74.5412	114.3819	39.8407
76.6394	115.8376	39.1981	75.627	115.0772	39.4503	74.4762	114.3905	39.9143
76.5485	115.8618	39.3133	75.4227	115.0478	39.6251	74.4702	114.3775	39.9074
76.5667	115.8384	39.2717	75.4357	115.0348	39.5991	74.5681	114.2879	39.7198
76.6325	115.8185	39.186	75.4098	115.014	39.6043	74.5403	114.3169	39.7766
76.5502	115.835	39.2847	75.3457	115.0114	39.6658	74.4503	114.278	39.8277
76.3961	115.8081	39.412	75.3968	114.9829	39.5861	74.4814	114.2373	39.7558
76.455	115.7617	39.3067	75.3223	114.95	39.6277	74.5048	114.2425	39.7376
76.4801	115.7371	39.257	75.389	114.9274	39.5385	74.9594	113.8995	38.9401
76.3675	115.7501	39.3826	75.3618	114.8887	39.5269	75.5015	113.4813	37.9797
76.416	115.7146	39.2986	75.3552	114.9162	39.561	75.7613	113.3444	37.5831
76.2939	115.6843	39.3904	75.3691	114.9144	39.5454	75.6684	113.3445	37.6761
76.2636	115.6756	39.412	75.3639	114.8928	39.5289	75.654	113.3271	37.6732
76.3727	115.66	39.2873	75.2773	114.898	39.6207	75.7406	113.3081	37.5675
76.4299	115.6098	39.18	75.1197	114.8625	39.7428	75.7787	113.2769	37.4982
76.4195	115.5544	39.1349	75.2877	114.8374	39.5497	75.7605	113.2708	37.5104
76.2313	115.597	39.3657	75.2877	114.8287	39.5411	75.6314	113.2864	37.655
76.1909	115.5501	39.3592	75.3041	114.8183	39.5142	75.5821	113.3263	37.7442
76.048	115.5561	39.5082	75.25	114.7918	39.5419	75.5803	113.2795	37.6991
76.0774	115.5215	39.4441	75.1439	114.749	39.6051	75.3187	113.3022	37.9836
76.048	115.499	39.451	75.0807	114.756	39.6753	74.9231	113.302	38.3789
75.9977	115.5146	39.5168	75.0495	114.7257	39.6762	74.7456	113.3401	38.5946
75.9796	115.5042	39.5246	75.0105	114.7317	39.7212	74.5793	113.373	38.7937
75.977	115.486	39.509	75.0582	114.6798	39.6216	74.5983	113.3193	38.721
75.9106	115.4992	39.5886	75.0738	114.6884	39.6147	74.7464	113.2501	38.5036
75.9267	115.4765	39.5497	74.9785	114.6417	39.6632	74.5395	113.2994	38.76
76.0636	115.4869	39.4233	75.1055	114.657	39.5516	74.3914	113.3358	38.9444
75.9345	115.447	39.5125	74.9187	114.6555	39.7368	74.4295	113.2795	38.85
76.048	115.389	39.341	74.8356	114.6347	39.7991	74.784	113.0459	38.2619
76.1502	115.4055	39.2553	74.8365	114.6321	39.7957	74.8157	113.0171	38.2014
75.9267	115.4046	39.4778	74.7906	114.6252	39.8346	74.7897	113.0067	38.217
75.7856	115.415	39.6294	74.8062	114.6174	39.8112	74.3368	113.1635	38.8266
75.8287	115.3662	39.5375	74.8832	114.5836	39.7004	74.2502	113.1513	38.9011
75.7856	115.3457	39.5601	74.7611	114.5914	39.8303	74.2156	113.1505	38.9349
75.7882	115.3414	39.5532	74.85	114.5786	39.7286	74.3446	113.1098	38.7652
75.757	115.3388	39.5818	74.6668	114.5984	39.9316	74.5282	113.0665	38.5383
75.8471	115.3336	39.4865	74.775	114.5559	39.7809	74.3074	113.0573	38.7499
75.7709	115.3232	39.5523	74.7568	114.5611	39.8043	74.1896	113.0717	38.8821
75.615	115.2401	39.6251	74.6962	114.5222	39.826	74.1125	113.0465	38.934
75.6323	115.2522	39.6199	74.678	114.5213	39.8433	74.1879	112.9885	38.8007
75.7151	115.1856	39.4705	74.6486	114.4546	39.806	74.4	112.9504	38.5504

74.3931	112.9859	38.5928
73.9731	113.0102	39.0371
73.78	113.0673	39.2873
74.0748	113.215	39.1402
79.1499	118.1142	38.9643
79.6461	118.6459	38.9998
79.6487	118.581	38.9323
79.685	118.4918	38.8067
79.6487	118.4563	38.8076
79.3006	118.5151	39.2146
79.298	118.4805	39.1826
79.244	118.4335	39.1895
79.1698	118.4606	39.2908
79.136	118.4528	39.3168
79.0624	118.3549	39.2925
78.975	118.3013	39.3263
78.8667	118.361	39.4943
78.8451	118.2857	39.4406
78.8407	118.2415	39.4008
78.8246	118.2159	39.3912
78.7429	118.1766	39.4337
78.7879	118.148	39.3601
78.7065	118.1393	39.4328
78.6467	118.1168	39.4701
78.6433	118.0475	39.4042
78.5827	118.064	39.4813
78.4813	118.0215	39.5402
78.393	118.0415	39.6484
78.3305	117.9639	39.6335
78.3896	117.9427	39.5532
78.4086	117.922	39.5134
78.3922	117.9116	39.5194
78.4762	117.76	39.2839
78.5229	117.7107	39.1877
78.49	117.6778	39.1877
78.7256	117.5816	38.8561
78.541	117.6028	39.0618
78.5983	117.5349	38.9366
78.5385	117.5002	38.9617
78.3497	117.4691	39.1193
78.2839	117.4907	39.2068
78.1324	117.5565	39.4242
78.1228	117.5357	39.4129
78.0969	117.5054	39.4086
78.016	117.5182	39.5022
78.0501	117.4439	39.3938
78.0345	117.4829	39.4484

77.8691	117.4942	39.6251
77.7842	117.5124	39.7281
77.8431	117.4872	39.6441
77.8094	117.4717	39.6623
77.8483	117.4587	39.6103
77.9596	117.3685	39.4089
77.9176	117.3565	39.4389
77.8665	117.3807	39.5142
77.8388	117.3573	39.5185
77.7496	117.3842	39.6346
77.799	117.3877	39.5887
77.7375	117.3444	39.6069
77.7522	117.3288	39.5766
77.7579	117.2918	39.5339
77.6682	117.3089	39.6406
77.6699	117.2396	39.5696
77.6067	117.1989	39.5922
77.7011	117.1634	39.4623
77.7505	117.1244	39.3739
77.6007	117.1071	39.5064
77.508	117.0915	39.5835
77.5068	117.0645	39.5577
77.4673	117.0517	39.5844
77.5071	117.0274	39.5203
77.4803	117.0274	39.5471
77.4179	117.0006	39.5826
77.411	116.9529	39.5419
77.5487	116.927	39.3783
77.5348	116.8196	39.2847
77.5324	116.7853	39.2529
77.4275	116.8083	39.3809
77.4465	116.8585	39.412
77.3928	116.9096	39.5168
77.4379	116.9192	39.4813
77.2846	116.8508	39.5662
77.4275	116.9183	39.4908
77.3702	116.8891	39.5189

Table A.2: 31 Hour Test

minimum mmHg	maximum mmHg	height mmHg
82.6138	122.2189	39.6051
82.6008	122.1999	39.5991
82.5618	122.1964	39.6346
82.5679	122.1938	39.6259
82.579	122.1799	39.6009
82.528	122.1955	39.6675
82.4683	122.2293	39.761
82.4717	122.1999	39.7281
82.4977	122.2639	39.7662
82.5254	122.2518	39.7264
82.586	122.2302	39.6441
82.5852	122.2293	39.6441
82.8138	122.2042	39.3904
82.6068	122.1574	39.5506
82.6432	122.1323	39.4891
82.6146	122.1323	39.5177
82.6181	122.1661	39.548
82.5713	122.1782	39.6069
82.5332	122.1929	39.6597
82.5254	122.1895	39.664
82.5042	122.1747	39.6705
82.4527	122.1903	39.7376
82.4466	122.186	39.7394
82.4674	122.1756	39.7082
82.483	122.1592	39.6762
82.6181	122.1635	39.5454
82.6337	122.1938	39.5601
82.6259	122.1609	39.535
82.6198	122.1747	39.5549
82.5755	122.1702	39.5947
82.4899	122.1895	39.6995
82.515	122.1869	39.6718
82.522	122.2128	39.6909
82.4986	122.244	39.7454
82.5497	122.2596	39.7099
82.5549	122.2501	39.6952
82.5142	122.2544	39.7402
82.4945	122.2548	39.7603
82.4475	122.3124	39.8649
82.4544	122.3055	39.8511
82.4917	122.2761	39.7844
82.5653	122.2613	39.6961
82.5176	122.2501	39.7324
82.4657	122.2778	39.8121
82.4639	122.2743	39.8104
82.4513	122.3138	39.8625
82.4458	122.2769	39.8312
82.4458	122.283	39.8372
82.5289	122.2596	39.7307
82.5791	122.2674	39.6883
82.6345	122.257	39.6225
82.6371	122.2761	39.6389
82.6458	122.2709	39.6251
82.6046	122.2425	39.6379
82.606	122.2778	39.6718
82.6008	122.2709	39.6701
82.6112	122.2648	39.6536
82.6086	122.2657	39.6571
82.7082	122.2206	39.5125
82.7592	122.2059	39.4467
82.7662	122.2302	39.464
82.7632	122.2337	39.4705
82.7575	122.2224	39.4649
82.7211	122.2371	39.516
82.7419	122.2397	39.4978
82.7696	122.2414	39.4718
82.7783	122.244	39.4657
82.7402	122.2622	39.522
82.7021	122.2423	39.5402
82.6751	122.2742	39.5991
82.6856	122.2787	39.593
82.6683	122.296	39.6277
82.6103	122.3159	39.7056
82.6077	122.3133	39.7056
82.6259	122.3739	39.748
82.6666	122.3471	39.6805
82.6406	122.3635	39.7229
82.6319	122.3488	39.7169
82.6301	122.3632	39.733
82.6389	122.3886	39.7498
82.6752	122.3436	39.6684
82.6536	122.3332	39.6796
82.6155	122.3367	39.7212
82.6354	122.3635	39.7281
82.6389	122.3618	39.7229
82.703	122.3367	39.6337
82.6953	122.3905	39.6951
82.7012	122.4094	39.7082
82.7185	122.3886	39.6701
82.6527	122.3947	39.742
82.6164	122.4137	39.7974
82.6744	122.3782	39.7039
82.6086	122.4129	39.8043
82.6285	122.483	39.8545
82.6548	122.3288	39.674
82.7307	122.2501	39.5194
82.7514	122.2423	39.4908
82.7177	122.2163	39.4986
82.7073	122.2189	39.5116
82.6726	122.2016	39.5289
82.6475	122.1418	39.4943
82.7263	122.0665	39.3402
82.698	122.1068	39.4089
82.7159	122.1366	39.4207
82.7549	122.1366	39.3817
82.735	122.1236	39.3887
82.7229	122.0994	39.3765
82.7705	122.0907	39.3202
82.7921	122.0873	39.2951
82.8692	122.037	39.1678
82.8565	122.0311	39.1745
82.8848	122.0284	39.1436
82.8753	122.044	39.1687
82.8398	122.076	39.2362
82.7991	122.1046	39.3055
82.7774	122.1176	39.3402
82.7402	122.1678	39.4276
82.683	122.1635	39.4804
82.6539	122.1544	39.5005
82.6562	122.2007	39.5445
82.6718	122.3133	39.6415
82.7237	122.3375	39.6138
82.6389	122.3592	39.7203
82.6164	122.3722	39.7558
82.638	122.3644	39.7264
82.6787	122.4259	39.7472
82.7385	122.4241	39.6857
82.7579	122.3966	39.6388
82.735	122.4146	39.6796
82.7151	122.4034	39.6883
82.7012	122.3904	39.6891
82.7177	122.4034	39.6857
82.7402	122.393	39.6528
82.7792	122.4354	39.6562
82.8138	122.4319	39.6181
82.809	122.4054	39.5965
82.7644	122.412	39.6476
82.7489	122.4103	39.6614

82.7558	122.4657	39.7099	82.851	122.4527	39.6017	82.7748	122.5791	39.8043
82.7748	122.4596	39.6848	82.9255	122.4319	39.5064	82.7306	122.6521	39.9215
82.7913	122.4709	39.6796	82.864	122.4501	39.5861	82.7419	122.6129	39.871
82.7826	122.4544	39.6718	82.8259	122.4778	39.6519	82.7142	122.6276	39.9134
82.8588	122.4302	39.5714	82.7896	122.4718	39.6822	82.7653	122.6224	39.8571
82.8266	122.4372	39.6106	82.7991	122.4501	39.651	82.7913	122.632	39.8407
82.9177	122.4008	39.483	82.8199	122.4588	39.6389	82.8406	122.6432	39.8026
82.9298	122.4189	39.4891	82.8803	122.4336	39.5533	82.8242	122.6839	39.8597
82.9125	122.4259	39.5134	82.935	122.4302	39.4952	82.8424	122.6476	39.8052
82.864	122.4562	39.5922	83.0104	122.4163	39.406	82.9684	122.6045	39.6361
82.8675	122.4163	39.5489	82.9783	122.4111	39.4328	83.0615	122.593	39.5315
82.8337	122.4068	39.5731	82.9212	122.4172	39.496	83.1394	122.5705	39.4311
82.858	122.406	39.548	82.9047	122.4363	39.5315	83.1992	122.5878	39.3887
82.8689	122.3359	39.467	82.9194	122.4389	39.5194	83.1507	122.6025	39.4519
82.8147	122.3791	39.5644	82.8935	122.4293	39.5359	83.1983	122.6285	39.4302
82.8398	122.4181	39.5783	82.8601	122.4416	39.5815	83.1896	122.6432	39.4536
82.7887	122.4441	39.6554	82.948	122.4189	39.4709	83.1446	122.6744	39.5298
82.8181	122.4562	39.6381	82.9498	122.412	39.4623	83.1358	122.6768	39.541
82.7904	122.4181	39.6277	83.0286	122.406	39.3774	83.1429	122.7038	39.561
82.7939	122.3938	39.5999	82.974	122.4137	39.4397	83.1022	122.7168	39.6147
82.7887	122.4086	39.6199	83.0952	122.3947	39.2995	83.0684	122.7298	39.6614
82.7491	122.3861	39.637	83.0892	122.4285	39.3393	83.0805	122.7307	39.6502
82.8277	122.4008	39.5731	83.1758	122.412	39.2362	83.0632	122.7064	39.6432
82.7939	122.4068	39.6129	83.1648	122.3949	39.23	83.0104	122.7567	39.7463
82.7973	122.4016	39.6043	83.1048	122.4146	39.3098	82.9705	122.7749	39.8043
82.8822	122.3505	39.4683	83.09	122.457	39.367	83.0072	122.793	39.7859
82.9151	122.341	39.4259	83.0424	122.4995	39.4571	83.0762	122.7896	39.7134
82.9818	122.341	39.3592	82.9653	122.5003	39.535	83.142	122.7402	39.5982
83.0199	122.3142	39.2943	82.8943	122.5471	39.6528	83.2303	122.6718	39.4415
83.016	122.3147	39.2987	82.8632	122.5999	39.7368	83.3429	122.6467	39.3038
83.0459	122.3566	39.3107	82.9593	122.5592	39.5999	83.3178	122.6597	39.3419
83.0407	122.3254	39.2847	83.0028	122.5526	39.5498	83.3611	122.6675	39.3064
82.9991	122.3704	39.3713	83.0822	122.5211	39.4389	83.3368	122.6779	39.341
82.9844	122.3765	39.3921	83.0632	122.5038	39.4406	83.2776	122.6741	39.3965
83.0017	122.4224	39.4207	82.9844	122.5073	39.5229	83.278	122.7194	39.4415
82.9939	122.3982	39.4042	82.9411	122.5471	39.606	83.2442	122.7038	39.4597
82.9922	122.4112	39.419	82.9368	122.5194	39.5826	83.1498	122.7021	39.5523
82.9411	122.4163	39.4752	82.9515	122.5333	39.5818	83.0727	122.7298	39.6571
82.8882	122.4336	39.5454	82.9896	122.5177	39.5281	82.9965	122.7567	39.7602
82.8692	122.4467	39.5774	82.8671	122.5658	39.6987	83.0381	122.7636	39.7255
82.8268	122.4259	39.5991	82.7168	122.6025	39.8857	83.0831	122.7445	39.6614
82.819	122.464	39.645	82.7185	122.6216	39.903	83.0908	122.7323	39.6414
82.7973	122.483	39.6857	82.7341	122.5843	39.8502	83.0805	122.7402	39.6597
82.7489	122.4865	39.7376	82.7644	122.5939	39.8294	83.058	122.7333	39.6753
82.754	122.496	39.742	82.8199	122.5532	39.7333	83.0485	122.7558	39.7073
82.8129	122.4752	39.6623	82.8658	122.5696	39.7039	83.0857	122.7523	39.6666
82.8222	122.4601	39.6379	82.8415	122.5688	39.7272	83.1437	122.761	39.6173

83.2658	122.7298	39.464	83.9551	122.5272	38.572	83.6789	122.7203	39.0414
83.3775	122.6839	39.3064	84.0054	122.49	38.4846	83.6434	122.7402	39.0968
83.323	122.6467	39.3237	83.9829	122.5255	38.5426	83.6798	122.7671	39.0873
83.3771	122.6371	39.26	83.9785	122.5358	38.5573	83.6486	122.8104	39.1618
83.3672	122.6354	39.2683	84.0374	122.5497	38.5123	83.6876	122.793	39.1055
83.3316	122.6554	39.3237	83.8801	122.5156	38.6354	83.8382	122.7766	38.9383
83.2563	122.6874	39.4311	83.9863	122.4874	38.501	83.9057	122.8124	38.9067
83.2173	122.7151	39.4978	84.0556	122.5229	38.4673	83.6754	122.8303	39.1548
83.1792	122.7099	39.5307	84.0036	122.541	38.5374	83.7551	122.8277	39.0726
83.1784	122.6935	39.5151	84.0391	122.5514	38.5123	83.5802	122.8355	39.2553
83.1403	122.7151	39.5748	83.8495	122.5497	38.7002	83.6806	122.8052	39.1245
83.164	122.7023	39.5383	83.7664	122.5436	38.7773	83.7413	122.7861	39.0449
83.1792	122.7073	39.5281	83.9015	122.548	38.6465	83.5906	122.8571	39.2665
83.2113	122.7047	39.4934	84.0079	122.5112	38.5033	83.6659	122.8285	39.1626
83.1697	122.7281	39.5584	83.7646	122.561	38.7963	83.6893	122.8623	39.173
83.1619	122.7532	39.5913	83.8209	122.5575	38.7366	83.6581	122.83	39.1719
83.155	122.7679	39.6129	83.7309	122.5393	38.8085	83.7923	122.806	39.0137
83.1671	122.7272	39.5601	83.7413	122.5766	38.8353	83.7075	122.8398	39.1323
83.1377	122.7679	39.6303	83.7109	122.58	38.8691	83.5265	122.8537	39.3272
83.1331	122.764	39.6308	83.6988	122.5982	38.8994	83.5689	122.8294	39.2605
83.2321	122.7506	39.5185	83.6997	122.6051	38.9054	83.5689	122.8597	39.2908
83.2295	122.7203	39.4908	83.8202	122.5534	38.7332	83.6962	122.7948	39.0986
83.2373	122.7246	39.4874	83.6971	122.5566	38.8595	83.7248	122.8026	39.0778
83.3498	122.6839	39.3341	83.6659	122.5783	38.9124	83.903	122.6997	38.7966
83.4001	122.6718	39.2717	83.7655	122.5705	38.805	83.5438	122.7818	39.238
83.3966	122.6484	39.2518	83.73	122.5696	38.8396	83.6027	122.7394	39.1367
83.9525	122.3852	38.4326	83.7326	122.5791	38.8466	83.5819	122.7783	39.1964
83.9339	122.4028	38.4689	83.8833	122.5722	38.6889	83.8452	122.7792	38.934
83.9196	122.4103	38.4906	83.9196	122.5722	38.6526	83.7499	122.819	39.0691
83.8703	122.451	38.5807	83.8995	122.5737	38.6742	83.7759	122.8242	39.0483
83.7889	122.4683	38.6794	83.8989	122.6242	38.7253	83.8911	122.7593	38.8682
83.7499	122.4882	38.7383	83.7257	122.6528	38.9271	83.8114	122.7481	38.9367
83.8209	122.5159	38.695	83.704	122.6579	38.9539	84.1058	122.7038	38.598
83.7508	122.5081	38.7574	83.7066	122.6952	38.9886	83.95	122.6952	38.7452
83.9266	122.4415	38.5149	83.665	122.6857	39.0206	83.8244	122.7627	38.9383
83.9488	122.438	38.4892	83.7975	122.6935	38.8959	83.8469	122.7766	38.9297
83.8408	122.4484	38.6075	83.6451	122.7151	39.07	83.8867	122.7636	38.8769
83.8157	122.49	38.6742	83.822	122.7102	38.8882	83.8772	122.7497	38.8725
83.9144	122.5029	38.5885	83.6798	122.7671	39.0873	84.1673	122.6172	38.4499
83.9205	122.5185	38.598	83.7205	122.7316	39.0111	84.347	122.5252	38.1782
83.8053	122.5168	38.7115	83.7187	122.7679	39.0492	84.5215	122.4224	37.9009
83.9361	122.47	38.5339	83.6728	122.7688	39.096	84.4479	122.4839	38.036
83.8668	122.4692	38.6024	83.7447	122.7636	39.0189	84.5102	122.4441	37.9338
83.9497	122.4671	38.5174	83.7404	122.7541	39.0137	84.4427	122.4319	37.9892
83.8209	122.5281	38.7071	83.8841	122.748	38.8639	84.4436	122.4519	38.0083
83.8088	122.5047	38.6959	83.829	122.7349	38.9059	84.4418	122.4562	38.0144
83.9283	122.5029	38.5746	83.5854	122.7428	39.1574	84.3457	122.5315	38.1858

84.1788	122.6089	38.4302	83.0467	123.1091	40.0624	82.8051	123.3741	40.569
84.1179	122.6207	38.5028	82.9775	123.1091	40.1317	82.9601	123.3299	40.3698
84.1725	122.5679	38.3954	82.9499	123.1472	40.1973	83.0052	123.3577	40.3525
83.7785	122.7047	38.9262	82.9584	123.1862	40.2278	83.1082	123.284	40.1758
83.9101	122.6987	38.7885	82.9437	123.181	40.2373	83.0627	123.2872	40.2246
83.7698	122.7653	38.9955	82.9316	123.1706	40.239	83.045	123.3092	40.2641
83.7984	122.7489	38.9505	83.1429	123.0693	39.9264	83.0251	123.3282	40.3031
83.7776	122.7506	38.973	83.0476	123.0927	40.0451	83.0043	123.3334	40.3291
83.7639	122.7472	38.9834	83.0623	123.0771	40.0147	82.9957	123.3629	40.3672
83.9041	122.6935	38.7894	83.2095	122.9965	39.787	83.013	123.3741	40.3611
83.943	122.722	38.779	83.3172	123.0053	39.6881	83.0086	123.3256	40.317
83.833	122.7376	38.9046	83.2771	122.9836	39.7065	82.9506	123.3724	40.4218
83.9378	122.6744	38.7366	83.2528	123.0061	39.7532	82.8759	123.3859	40.51
83.9326	122.6805	38.7478	83.1602	123.0753	39.9152	82.7445	123.4434	40.6989
83.7949	122.7108	38.9158	83.1307	123.0667	39.9359	82.8753	123.5456	40.6703
83.8001	122.6822	38.8821	83.1178	123.1005	39.9827	83.0407	123.3949	40.3542
83.8313	122.7437	38.9124	83.1065	123.1083	40.0018	83.0684	123.3031	40.2347
83.6247	122.8089	39.1842	83.1559	123.0883	39.9325	83.0649	123.2078	40.1429
83.5533	122.8822	39.3289	83.194	123.0667	39.8727	83.019	123.1975	40.1784
83.549	122.8952	39.3462	83.2485	123.0793	39.8308	82.9627	123.1758	40.2131
83.5481	122.9039	39.3557	83.1524	123.1446	39.9922	82.9186	123.1524	40.2338
83.5767	122.8623	39.2856	83.1749	123.1342	39.9593	83.1939	122.9587	39.7647
83.5334	122.8987	39.3653	83.1377	123.1585	40.0208	83.4563	122.7965	39.3402
83.5793	122.8563	39.2769	83.0909	123.142	40.0511	83.6001	122.7073	39.1072
83.446	122.9437	39.4978	83.0675	123.1412	40.0736	83.8893	122.606	38.7167
83.4767	122.912	39.4353	83.0623	123.155	40.0927	84.1067	122.3696	38.2629
83.5802	122.8389	39.2588	83.2745	123.0953	39.8208	84.2288	122.2795	38.0507
83.5412	122.8857	39.3445	83.3886	123.0881	39.6996	84.3734	122.1687	37.7953
83.452	122.8866	39.4345	83.4598	123.0554	39.5956	84.4695	122.0916	37.6221
83.4321	122.8848	39.4527	83.3628	123.065	39.7021	84.2765	122.2028	37.9263
83.3879	122.8952	39.5073	83.3083	123.0961	39.7879	84.4392	122.1089	37.6697
83.4191	122.8918	39.4727	83.2676	123.1325	39.8649	84.4583	122.1323	37.674
83.3637	122.9351	39.5714	83.2451	123.136	39.8909	84.5327	122.0942	37.5615
83.3084	122.9322	39.6238	83.2494	123.1689	39.9195	84.3111	122.1947	37.8836
83.2806	122.9472	39.6666	83.2451	123.1351	39.89	84.2885	122.2172	37.9286
83.3065	122.9264	39.6199	83.2406	123.1613	39.9207	84.305	122.2241	37.9191
83.2849	122.9359	39.651	83.2537	123.1749	39.9212	84.4254	122.173	37.7476
83.2658	122.9541	39.6883	83.174	123.2018	40.0277	84.3083	122.1835	37.8752
83.1836	122.9853	39.8017	83.129	123.2018	40.0728	84.3258	122.1635	37.8377
83.1255	123.0234	39.8978	83.0415	123.2537	40.2122	84.4436	122.0933	37.6498
83.1411	123.0572	39.916	83.0407	123.2485	40.2079	84.5172	122.0526	37.5355
83.2062	123.0441	39.8379	83.0078	123.2633	40.2555	84.5648	122.031	37.4662
83.2234	123.0554	39.832	82.9801	123.2797	40.2997	84.6228	121.9548	37.332
83.3568	123.0061	39.6493	82.9067	123.3163	40.4096	84.7068	121.9548	37.248
83.3663	122.9714	39.6051	82.7757	123.3923	40.6166	84.7804	121.8595	37.0791
83.3013	123.0026	39.7013	82.8207	123.362	40.5413	84.7267	121.8637	37.137
83.1948	123.0381	39.8433	82.8069	123.4027	40.5958	84.7709	121.8742	37.1034

84.7553	121.8708	37.1155	84.7989	122.2962	37.4973	84.9467	122.5298	37.5831
84.6869	121.9375	37.2506	84.492	122.4086	37.9165	84.9138	122.5177	37.6039
84.7432	121.824	37.0808	84.744	122.3081	37.5641	84.9434	122.5235	37.5801
84.7562	121.8379	37.0817	84.4557	122.4467	37.991	85.0116	122.5107	37.4991
84.8289	121.8084	36.9795	84.7241	122.3523	37.6281	84.9623	122.5246	37.5623
84.6774	121.8942	37.2168	84.6003	122.3635	37.7632	84.9614	122.5237	37.5623
84.6333	121.8892	37.2559	84.5674	122.3886	37.8212	84.9129	122.5367	37.6238
84.5908	121.9522	37.3614	84.4522	122.4501	37.9979	84.9112	122.5125	37.6013
84.5388	122.0024	37.4636	84.1955	122.5667	38.3712	84.8523	122.5073	37.655
84.4184	122.0994	37.681	84.1872	122.5462	38.359	84.9068	122.4709	37.5641
84.4643	122.0535	37.5892	84.2426	122.4796	38.2369	85.0235	122.3887	37.3652
84.2704	122.1531	37.8827	84.0651	122.5289	38.4638	85.0454	122.4224	37.377
84.2756	122.1921	37.9165	84.2634	122.477	38.2135	84.9441	122.47	37.526
84.1491	122.2934	38.1443	84.1006	122.5073	38.4066	84.7466	122.5809	37.8342
84.2034	122.2205	38.017	84.1699	122.4596	38.2897	84.6228	122.6787	38.0559
84.2401	122.173	37.933	84.4687	122.3289	37.8602	84.5128	122.7601	38.2473
84.2357	122.1947	37.9589	84.4192	122.3552	37.936	84.5812	122.6727	38.0914
84.3448	122.1141	37.7693	84.2963	122.3834	38.0871	84.6081	122.6753	38.0672
84.1024	122.2284	38.1261	84.3613	122.3531	37.9918	84.7055	122.6512	37.9457
83.9465	122.3185	38.372	84.434	122.3453	37.9113	84.7475	122.619	37.8715
83.8088	122.4086	38.5998	84.1223	122.4596	38.3374	84.699	122.6458	37.9468
83.7603	122.4215	38.6612	84.1179	122.5324	38.4144	84.6427	122.6969	38.0542
83.7941	122.4562	38.6621	84.2236	122.4856	38.262	84.3024	122.8441	38.5417
83.6388	122.5094	38.8706	84.1491	122.5627	38.4136	84.2712	122.8225	38.5513
83.5516	122.5921	39.0405	84.176	122.5393	38.3633	84.2097	122.8649	38.6552
83.5793	122.606	39.0267	84.1611	122.5358	38.3747	84.5431	122.6995	38.1564
83.5629	122.6614	39.0986	84.3414	122.4804	38.1391	84.4115	122.748	38.3365
83.7057	122.5861	38.8803	83.9474	122.6398	38.6924	84.2633	122.8019	38.5385
83.6858	122.606	38.9202	84.0348	122.6121	38.5772	84.2262	122.7827	38.5565
84.1145	122.3878	38.2733	84.0954	122.6467	38.5513	84.3007	122.7766	38.4759
83.4693	122.6346	39.1652	84.1405	122.6034	38.4629	84.3734	122.7636	38.3902
83.1913	122.7728	39.5815	84.3561	122.7411	38.385	84.4591	122.7038	38.2447
83.5603	122.677	39.1167	84.3561	122.9177	38.5617	84.3483	122.7896	38.4413
83.5759	122.5835	39.0076	84.4941	122.8908	38.3967	84.3189	122.7463	38.4274
83.7161	122.651	38.9349	84.5743	122.7601	38.1858	84.363	122.7116	38.3486
83.5282	122.6831	39.1548	84.8436	122.5497	37.7061	84.3699	122.7226	38.3527
83.5499	122.7168	39.167	84.8904	122.5558	37.6654	84.2747	122.7939	38.5192
83.8469	122.5774	38.7305	84.7787	122.6285	37.8498	84.3076	122.8242	38.5166
84.3769	122.3843	38.0074	84.9727	122.4848	37.5121	84.3266	122.7974	38.4707
84.4351	122.3376	37.9025	85.0411	122.4137	37.3727	84.2522	122.8337	38.5816
84.253	122.477	38.2239	85.0212	122.4458	37.4246	84.0556	122.9506	38.895
84.1102	122.4718	38.3616	85.0482	122.4433	37.3951	84.2054	122.8641	38.6586
83.9803	122.5558	38.5755	85.0497	122.4596	37.4099	84.3708	122.8407	38.4699
83.691	122.6761	38.9851	84.8852	122.5705	37.6853	84.0087	122.9904	38.9816
83.3689	122.7653	39.3964	84.7917	122.5722	37.7805	83.8815	123.0372	39.1557
84.4271	122.4302	38.0031	84.8826	122.5021	37.6195	83.7482	123.1619	39.4138
84.6704	122.3107	37.6403	84.9441	122.5003	37.5563	83.9586	123.0346	39.076

83.6893	123.1481	39.4588	85.4914	122.406	36.9146	85.723	122.5455	36.8225
83.4988	123.2442	39.7454	85.3511	122.47	37.1189	85.72	122.5662	36.8462
83.4901	123.2598	39.7697	85.3191	122.4631	37.1441	85.6784	122.5904	36.912
83.5031	123.2728	39.7697	85.3095	122.4397	37.1302	85.6724	122.5852	36.9128
83.6079	123.2291	39.6212	85.3294	122.4553	37.1259	85.7174	122.5679	36.8505
83.6434	123.2537	39.6103	85.2515	122.5203	37.2688	85.6836	122.5817	36.8981
83.7118	123.233	39.5211	85.198	122.5173	37.3194	85.6481	122.6276	36.9795
83.756	123.2174	39.4614	85.1969	122.5601	37.3631	85.6923	122.5991	36.9068
83.6702	123.2693	39.5991	85.0913	122.645	37.5537	85.693	122.5931	36.9
83.7603	123.2208	39.4605	85.177	122.6198	37.4428	85.7226	122.5566	36.834
83.9292	123.1446	39.2155	85.2368	122.6086	37.3718	85.7009	122.5714	36.8704
83.9586	123.1299	39.1713	85.2957	122.5549	37.2592	85.7087	122.535	36.8262
83.9048	123.1542	39.2494	85.3121	122.5636	37.2514	85.6862	122.5843	36.8981
83.6018	123.3066	39.7047	85.4983	122.7212	37.2229	85.7538	122.5921	36.8384
83.9153	123.1784	39.2631	85.3213	122.6988	37.3775	85.7815	122.5428	36.7613
84.04	123.1325	39.0925	85.2351	122.6484	37.4134	85.7183	122.5965	36.8782
83.9387	123.2338	39.2951	85.2931	122.5384	37.2454	85.6913	122.5975	36.9062
84.2885	123.0346	38.7461	85.1944	122.5159	37.3216	85.6784	122.6354	36.957
83.8815	123.1862	39.3047	85.171	122.4319	37.261	85.6836	122.6103	36.9267
83.9075	123.2087	39.3012	85.158	122.4016	37.2436	85.7113	122.5947	36.8834
83.9524	123.1384	39.186	85.1095	122.4267	37.3173	85.8291	122.5748	36.7457
83.885	123.1645	39.2795	84.9623	122.4648	37.5026	85.8239	122.6086	36.7847
83.833	123.2191	39.3861	85.0438	122.3746	37.3308	85.856	122.5791	36.7232
83.8426	123.1983	39.3557	84.977	122.367	37.39	85.8802	122.5817	36.7015
83.8157	123.2468	39.4311	84.8549	122.4172	37.5623	85.8305	122.5966	36.7662
83.9188	123.1897	39.2709	84.7544	122.4458	37.6914	85.8282	122.5973	36.7691
83.9889	123.1619	39.173	84.6826	122.4406	37.758	85.7927	122.6173	36.8245
84.5232	122.8372	38.314	84.7103	122.4371	37.7269	85.8213	122.5965	36.7752
85.1164	122.5402	37.4238	84.6878	122.4198	37.7321	85.733	122.6121	36.8791
85.0482	122.5913	37.5431	84.6592	122.4588	37.7996	85.7694	122.6181	36.8488
85.2238	122.5211	37.2973	84.828	122.3394	37.5114	85.8395	122.6008	36.7613
85.261	122.5133	37.2523	84.893	122.3436	37.4506	85.8984	122.5705	36.6721
85.352	122.4215	37.0696	84.9917	122.4865	37.4948	86.0947	122.5781	36.4834
85.3546	122.4293	37.0748	85.2402	122.638	37.3978	86.1383	122.5246	36.3863
85.2835	122.4735	37.19	85.5459	122.4744	36.9284	86.2084	122.5722	36.3638
85.2203	122.509	37.2887	85.5381	122.4822	36.944	86.2785	122.5142	36.2357
85.2273	122.5211	37.2939	85.4221	122.5566	37.1345	86.2422	122.5333	36.2911
85.2473	122.5235	37.2762	85.4308	122.5428	37.112	86.1642	122.5774	36.4132
85.1909	122.5575	37.3666	85.4178	122.58	37.1622	86.2283	122.5748	36.3465
85.0575	122.651	37.5935	85.627	122.5138	36.8868	86.1997	122.6198	36.4201
85.1026	122.5445	37.442	85.6533	122.457	36.8037	86.1651	122.6432	36.4781
85.1614	122.5497	37.3883	85.5381	122.5324	36.9942	85.9952	122.7481	36.7529
85.1831	122.5384	37.3554	85.6143	122.4432	36.8288	86.0136	122.7203	36.7067
85.2177	122.4709	37.2532	85.5962	122.5211	36.925	85.9469	122.7593	36.8124
85.2515	122.4397	37.1882	85.6473	122.5774	36.9302	85.901	122.7177	36.8167
85.2605	122.4821	37.2216	85.7382	122.5272	36.789	85.8568	122.7333	36.8765
85.2671	122.5099	37.2428	85.7486	122.5116	36.763	85.9322	122.7151	36.7829

86.1183	122.6623	36.5439	86.5712	121.7877	35.2164	86.9852	121.3339	34.3487
86.0681	122.6883	36.6201	86.5938	121.8024	35.2086	86.9705	121.3703	34.3998
86.1714	122.6574	36.486	86.5686	121.7998	35.2311	86.9843	121.3754	34.3911
86.1634	122.6458	36.4825	86.5314	121.7954	35.264	87.0181	121.359	34.3409
86.1729	122.6242	36.4513	86.5105	121.8144	35.3039	87.1004	121.3114	34.211
86.1859	122.5982	36.4123	86.4526	121.7643	35.3117	87.1004	121.3373	34.237
86.101	122.6528	36.5517	86.4673	121.7348	35.2675	87.0672	121.3519	34.2847
86.1071	122.658	36.5509	86.3799	121.7374	35.3576	87.0458	121.3997	34.3539
86.1235	122.6441	36.5206	86.3435	121.7106	35.3671	86.993	121.3936	34.4007
86.1945	122.6631	36.4686	86.3045	121.7088	35.4043	86.9765	121.398	34.4214
86.1291	122.6495	36.5204	86.2751	121.6985	35.4234	87.0172	121.4672	34.45
86.0698	122.7038	36.634	86.2552	121.6985	35.4433	87.0397	121.5166	34.4769
86.0127	122.722	36.7093	86.1855	121.6981	35.5126	87.0311	121.5357	34.5046
86.0188	122.7168	36.6981	86.1807	121.7504	35.5697	87.0406	121.5807	34.5401
85.9642	122.7419	36.7778	86.2301	121.766	35.536	86.9554	121.5959	34.6405
86.0664	122.6883	36.6219	86.2214	121.74	35.5186	86.9358	121.5712	34.6353
86.0214	122.6961	36.6747	86.2387	121.74	35.5013	86.9358	121.5504	34.6146
86.0006	122.7186	36.718	86.2448	121.7599	35.5152	86.9375	121.5244	34.5868
86.0753	122.7032	36.6278	86.3166	121.7868	35.4701	86.928	121.5192	34.5912
86.1893	122.6952	36.5058	86.3132	121.7911	35.4779	86.8873	121.5469	34.6596
86.0387	122.7307	36.692	86.329	121.81	35.4809	86.8968	121.5504	34.6535
85.8984	122.8268	36.9284	86.3184	121.7521	35.4338	86.8683	121.5867	34.7185
85.843	122.8337	36.9908	86.256	121.7279	35.4719	86.8426	121.6364	34.7938
85.8707	122.7939	36.9232	86.2335	121.7054	35.4719	86.9029	121.6231	34.7202
86.8873	122.1366	35.2493	86.1547	121.7175	35.5628	86.8925	121.6352	34.7427
86.967	122.0596	35.0926	86.1599	121.7175	35.5576	86.896	121.637	34.741
86.9721	122.076	35.1039	86.2119	121.7244	35.5126	86.8925	121.6179	34.7254
86.9886	122.0847	35.096	86.1677	121.7478	35.5801	86.9055	121.6248	34.7193
86.941	122.0786	35.1376	86.1317	121.7439	35.6122	86.9185	121.6188	34.7003
86.9254	122.0752	35.1497	86.1261	121.7392	35.613	86.9713	121.6223	34.6509
86.9583	122.0977	35.1393	86.1149	121.7877	35.6728	86.9642	121.6197	34.6555
86.8917	122.0985	35.2069	86.1634	121.7747	35.6113	86.9453	121.6197	34.6743
86.7635	121.9652	35.2017	86.1945	121.8284	35.6338	86.9644	121.6335	34.6691
86.7159	121.8578	35.1419	86.1989	121.8266	35.6277	86.9453	121.6508	34.7055
86.6109	121.8249	35.214	86.1287	121.8812	35.7524	86.909	121.695	34.786
86.534	121.7651	35.2311	86.1036	121.876	35.7724	86.9462	121.6604	34.7141
86.5262	121.7556	35.2294	86.1449	121.8804	35.7355	86.9713	121.6751	34.7038
86.5531	121.7175	35.1644	86.56	121.4811	34.9211	86.9298	121.6759	34.7462
86.6258	121.6985	35.0727	86.8587	121.2845	34.4258	86.8682	121.6664	34.7982
86.6223	121.7201	35.0978	86.8449	121.2958	34.4509	86.8319	121.6751	34.8432
86.6111	121.7591	35.148	86.8994	121.288	34.3885	86.8319	121.6396	34.8077
86.5634	121.753	35.1896	86.8951	121.2906	34.3955	86.838	121.6474	34.8094
86.5453	121.7495	35.2043	86.9731	121.3166	34.3435	86.8492	121.6326	34.7834
86.5272	121.7597	35.2325	87.0172	121.3122	34.295	86.8613	121.6361	34.7748
86.5461	121.7357	35.1896	87.0155	121.3356	34.3201	86.8527	121.6638	34.8111
86.5981	121.7357	35.1376	86.9668	121.3492	34.3824	86.8726	121.6924	34.8198
86.5678	121.753	35.1852	86.9869	121.3347	34.3478	86.8631	121.6933	34.8302

86.8206	121.7573	34.9367	80.3441	120.0817	39.7376	77.3642	117.2785	39.9143
86.5834	121.8483	35.2649	80.2297	120.012	39.7824	77.4153	117.1616	39.7463
86.579	121.8405	35.2614	79.9743	119.8453	39.871	77.5548	117.0387	39.4839
86.5747	121.8335	35.2588	79.9881	119.8028	39.8147	77.6178	116.8593	39.2415
86.6059	121.8483	35.2424	79.957	119.7812	39.8242	77.6595	116.7633	39.1037
86.5972	121.9245	35.3273	79.9396	119.7595	39.8199	77.469	116.7096	39.2406
86.5583	121.9262	35.368	79.9344	119.6747	39.7402	77.3469	116.6377	39.2908
86.4275	121.8136	35.3861	80.0184	119.6037	39.5852	77.3097	116.5693	39.2596
85.9458	121.6699	35.724	79.8894	119.5682	39.6788	77.0075	116.5156	39.5082
85.4039	121.6578	36.2538	79.7387	119.5725	39.8338	77.0023	116.5165	39.5142
85.1658	121.5019	36.3361	79.6492	119.5399	39.8907	76.8334	116.3944	39.561
85.2134	121.8214	36.608	79.5534	119.4937	39.9403	76.6013	116.3684	39.7671
85.1623	121.7764	36.6141	79.4348	119.414	39.9792	76.4057	116.2849	39.8793
85.022	121.7184	36.6964	79.4331	119.3118	39.8788	76.4177	116.1848	39.7671
84.9164	121.6716	36.7552	79.2694	119.3352	40.0658	76.4792	116.1052	39.6259
84.8523	121.5902	36.7379	79.2079	119.2313	40.0234	76.4195	116.0099	39.5904
84.7434	121.4893	36.7459	79.227	119.0893	39.8623	76.3848	115.9337	39.5489
84.6938	121.3893	36.6955	79.0598	119.0806	40.0208	76.4879	115.8298	39.3419
84.5327	121.3737	36.841	78.9119	118.9691	40.0572	76.3684	115.7778	39.4094
84.5466	121.2663	36.7197	78.7983	118.8988	40.1005	76.1614	115.6938	39.5324
84.4782	121.1971	36.7189	78.6935	118.794	40.1005	76.1291	115.715	39.5859
84.4306	121.1451	36.7145	78.5792	118.7403	40.1611	75.9648	115.6904	39.7255
84.3102	121.1027	36.7925	78.4762	118.6805	40.2044	75.8895	115.6557	39.7662
84.253	120.9875	36.7345	78.4051	118.6468	40.2416	75.77	115.6081	39.8381
84.118	120.9326	36.8146	78.3246	118.6078	40.2832	75.7076	115.6245	39.9169
84.0504	120.8152	36.7648	78.3142	118.5732	40.259	75.5639	115.5449	39.981
83.9378	120.7476	36.8098	78.2318	118.555	40.3232	75.4946	115.4756	39.981
83.8131	120.6541	36.841	78.1964	118.4978	40.3014	75.3578	115.4202	40.0624
83.7066	120.564	36.8574	78.1012	118.4649	40.3637	75.227	115.3697	40.1426
83.6044	120.4705	36.8661	78.0293	118.4251	40.3958	75.3413	115.2833	39.942
83.4919	120.3562	36.8643	77.9367	118.3697	40.433	75.3578	115.2634	39.9056
83.3836	120.228	36.8444	77.8457	118.3186	40.4728	75.3794	115.2106	39.8312
83.2617	120.123	36.8613	77.7574	118.2813	40.5239	75.3344	115.1838	39.8493
83.1567	120.015	36.8583	77.7366	118.232	40.4954	75.4124	115.0902	39.6779
83.0606	119.8617	36.8011	77.7147	118.1842	40.4695	75.0478	115.0599	40.0122
82.9783	119.7864	36.8081	77.7461	118.1125	40.3663	75.1396	115.0798	39.9403
82.8839	119.7673	36.8834	77.7946	118.0146	40.22	75.2896	114.9883	39.6987
82.8181	119.7448	36.9267	77.7314	117.9635	40.2321	75.1621	114.9543	39.7922
82.761	119.692	36.931	77.6292	117.9176	40.2884	75.2063	114.911	39.7047
82.6978	119.5569	36.8592	77.6076	117.8735	40.2659	75.2374	114.8408	39.6034
82.6892	119.4174	36.7283	77.5426	117.7704	40.2278	75.1699	114.8408	39.671
82.599	119.3361	36.7371	77.5374	117.7505	40.2131	74.8434	114.8235	39.9801
81.7764	119.5517	37.7753	77.5385	117.6812	40.1426	74.7845	114.7794	39.9948
81.3434	119.7604	38.417	77.4612	117.6466	40.1853	74.7508	114.7534	40.0026
80.9052	119.7232	38.818	77.3591	117.5609	40.2018	74.7029	114.7372	40.0343
80.8186	120.0332	39.2146	77.2716	117.5764	40.3048	74.7759	114.6806	39.9048
80.6723	119.9951	39.3228	77.1737	117.5375	40.3637	74.9075	114.6088	39.7013

74.8096	114.5516	39.742	72.5373	112.7911	40.2538	75.9856	115.2201	39.2345
74.7629	114.5048	39.742	72.5503	112.7305	40.1801	75.8551	115.2349	39.3798
74.6832	114.4719	39.7887	72.9313	112.5824	39.651	75.873	115.0409	39.1678
74.575	114.4719	39.897	72.4343	112.6603	40.226	75.8384	114.9863	39.1479
74.6217	114.3827	39.761	72.5252	112.7045	40.1793	76.3294	114.5577	38.2283
74.4483	114.3822	39.9339	72.7123	112.6517	39.9394	76.3069	114.5646	38.2577
74.4321	114.3758	39.9437	74.4355	114.0329	39.5974	76.0965	114.5733	38.4768
74.5057	114.3455	39.8398	78.1384	118.0749	39.9365	76.1025	114.4719	38.3694
74.4849	114.3126	39.8277	77.9557	118.0371	40.0814	76.2324	114.2832	38.0507
74.5221	114.2459	39.7238	77.87	117.9739	40.1039	76.0939	114.3276	38.2337
74.2727	114.252	39.9792	77.7167	117.9315	40.2148	76.0809	114.2225	38.1417
74.1134	114.1896	40.0762	77.6518	117.8553	40.2035	75.893	114.2087	38.3157
74.032	114.1316	40.0996	77.6266	117.7635	40.1369	75.983	113.9766	37.9936
74.1844	114.0407	39.8563	77.6916	117.6111	39.9195	75.9856	113.89	37.9044
74.1656	113.9946	39.829	77.4803	117.5756	40.0953	76.2272	113.6562	37.429
74.0199	113.9913	39.9714	77.5885	117.4699	39.8814	76.1597	113.5956	37.4359
74.0043	113.9359	39.9316	77.3409	117.5132	40.1724	75.6652	113.8943	38.2291
74.0251	113.8969	39.8719	77.1426	117.5184	40.3759	75.7424	113.6881	37.9457
73.8224	113.8632	40.0407	77.4413	117.166	39.7246	75.5855	113.7263	38.1408
73.7757	113.7965	40.0208	78.2415	116.623	38.3815	75.8886	113.8398	37.9511
73.8458	113.7679	39.9221	78.225	116.5174	38.2923	76.3121	114.0121	37.7
73.6839	113.7211	40.0373	78.2735	116.3987	38.1252	76.655	114.2728	37.6177
73.5701	113.6326	40.0625	78.1341	116.3805	38.2464	76.9711	114.4468	37.4757
73.515	113.6267	40.1117	77.9592	116.3719	38.4127	77.1668	114.7248	37.558
73.5141	113.5462	40.0321	77.8574	116.3325	38.4751	77.3824	114.9474	37.5649
73.3011	113.5358	40.2347	77.9375	116.1666	38.2291	77.6362	115.1552	37.519
73.2977	113.4934	40.1957	77.7808	116.1848	38.404	77.9851	115.3089	37.3238
73.2977	113.4094	40.1117	77.6388	116.1511	38.5123	78.1791	115.5094	37.3302
73.2396	113.3877	40.1481	77.5435	116.1571	38.6136	78.2865	115.738	37.4515
73.1331	113.3574	40.2243	77.4318	116.1086	38.6768	78.3809	116.0212	37.6403
72.9993	113.3463	40.347	77.4275	116.0662	38.6387	78.6017	116.171	37.5692
72.9617	113.2838	40.3222	77.3175	115.9986	38.6812	78.6935	116.416	37.7225
73.0578	113.2206	40.1628	77.2249	115.9467	38.7218	78.9169	116.4992	37.5822
72.9435	113.1808	40.2373	76.9979	115.9484	38.9505	79.2772	116.5329	37.2558
72.9062	113.1357	40.2295	76.7763	116.016	39.2397	79.4589	116.6364	37.1775
72.8638	113.1132	40.2494	76.6351	115.9857	39.3505	79.5751	116.7668	37.1917
72.8603	113.0795	40.2191	76.2636	116.1181	39.8545	79.6348	116.9954	37.3606
72.8837	113.0457	40.162	76.3389	116.0194	39.6805	79.8911	116.9564	37.0653
72.9728	112.941	39.9682	76.3052	115.9735	39.6684	79.9362	117.1391	37.2029
72.8075	112.9227	40.1152	76.0878	115.9389	39.8511	80.0895	117.2257	37.1363
72.7538	112.9097	40.1559	76.056	115.9705	39.9145	80.086	117.431	37.345
72.6871	112.8967	40.2096	76.3502	115.7181	39.3679	80.0687	117.6275	37.5589
72.7131	112.8811	40.168	76.7789	115.3318	38.553	80.5627	117.4997	36.937
73.0292	112.7556	39.7264	76.7156	115.2123	38.4967	80.9494	117.4136	36.4643
72.7582	112.8292	40.071	76.2939	115.3751	39.0812	80.9113	117.7245	36.8133
72.6075	112.8179	40.2105	76.2238	115.3604	39.1367	80.6731	118.0596	37.3865
72.5791	112.7842	40.2052	76.2792	115.1768	38.8976	81.0931	117.9237	36.8306

81.3079	117.954	36.6461	83.8763	120.2774	36.4011	84.3786	120.8368	36.4582
81.4534	117.98	36.5266	83.8885	120.3068	36.4184	84.3873	120.8308	36.4435
81.5625	118.0882	36.5258	83.937	120.2939	36.3569	84.4176	120.8463	36.4288
81.7131	118.0882	36.375	83.9448	120.3242	36.3794	84.376	120.8377	36.4617
81.766	118.1939	36.4279	84.0106	120.3207	36.3101	84.4106	120.8411	36.4305
81.8699	118.2302	36.3604	84.014	120.3433	36.3292	84.3942	120.8637	36.4695
81.9842	118.3125	36.3283	84.0348	120.3493	36.3145	84.3812	120.881	36.4998
82.037	118.4294	36.3924	84.0643	120.3562	36.2919	84.3981	120.9035	36.5054
81.9201	118.697	36.7769	84.0374	120.4333	36.3959	84.3864	120.9312	36.5448
82.0232	118.8269	36.8037	84.0166	120.4333	36.4166	84.4124	120.997	36.5846
82.0976	118.9204	36.8228	84.0625	120.4541	36.3915	84.3881	121.0533	36.6652
82.3209	118.8704	36.5494	84.1032	120.4523	36.3491	84.473	121.0039	36.531
82.2526	119.0304	36.7778	84.1344	120.5017	36.3673	84.4972	120.9788	36.4816
82.4735	118.9793	36.5058	84.1902	120.5247	36.3345	84.4418	120.9771	36.5353
82.638	118.988	36.35	84.2444	120.5545	36.3101	84.434	120.9918	36.5578
82.6813	119.0581	36.3768	84.2643	120.5415	36.2772	84.421	120.944	36.523
82.7705	119.1664	36.3959	84.2652	120.5571	36.2919	84.4193	121.0014	36.582
82.8675	119.2201	36.3526	84.3154	120.5632	36.2478	84.4132	121.0109	36.5976
82.9705	119.2287	36.2582	84.3223	120.5407	36.2183	84.421	120.9996	36.5786
83.0318	119.2201	36.1883	84.3076	120.5606	36.253	84.4773	120.9563	36.479
83.0294	119.2772	36.2478	84.2782	120.6134	36.3352	84.5206	120.9771	36.4565
83.0615	119.3032	36.2417	84.2404	120.619	36.3786	84.4998	120.9771	36.4773
83.1429	119.3551	36.2123	84.2582	120.6541	36.3959	84.7285	120.713	35.9845
83.2035	119.3837	36.1802	84.273	120.6324	36.3595	85.1196	120.4146	35.295
83.2416	119.4348	36.1932	84.3059	120.6558	36.35	85.0991	120.416	35.3169
83.265	119.4842	36.2192	84.331	120.6203	36.2893	85.0411	120.4073	35.3662
83.3109	119.5353	36.2244	84.3596	120.6671	36.3075	85.0315	120.4263	35.3948
83.3438	119.6106	36.2668	84.4158	120.6697	36.2538	85.0281	120.4038	35.3757
83.363	119.6799	36.3169	84.4548	120.7	36.2452	85.0497	120.3969	35.3472
83.4615	119.7249	36.2634	84.4219	120.7009	36.279	85.0142	120.4099	35.3957
83.4745	119.8072	36.3326	84.3875	120.7124	36.3248	84.9839	120.422	35.4381
83.5828	119.8089	36.2261	84.3873	120.6913	36.3041	84.9172	120.4593	35.542
83.6027	119.8531	36.2504	84.4132	120.7398	36.3266	84.9328	120.4657	35.5329
83.601	119.8678	36.2668	84.4479	120.7424	36.2945	84.8835	120.4913	35.6078
83.6261	119.8817	36.2556	84.4236	120.7485	36.3249	84.9138	120.519	35.6052
83.6728	119.8834	36.2105	84.3829	120.7502	36.3673	85.0688	120.4558	35.387
83.7198	119.9151	36.1953	84.3864	120.7589	36.3725	85.1155	120.4939	35.3783
83.749	119.9371	36.188	84.4202	120.7641	36.3439	85.1684	120.5	35.3316
83.8235	119.9665	36.143	84.4422	120.7397	36.2975	85.2047	120.5034	35.2987
83.846	120.0297	36.1837	84.3977	120.7701	36.3725	85.1623	120.5441	35.3818
83.8789	120.0652	36.1863	84.408	120.816	36.408	85.1169	120.5785	35.4615
83.9032	120.1007	36.1976	84.4098	120.8082	36.3985	85.074	120.6273	35.5533
83.9118	120.1466	36.2348	84.4418	120.8091	36.3673	85.0766	120.6134	35.5368
83.8893	120.1908	36.3015	84.4661	120.8437	36.3777	85.0671	120.6281	35.5611
83.8713	120.2164	36.3451	84.4366	120.8351	36.3985	85.1459	120.6047	35.4589
83.8668	120.2402	36.3733	84.4029	120.8403	36.4374	85.2169	120.5753	35.3584
83.9049	120.2185	36.3136	84.3585	120.8603	36.5019	85.1866	120.5684	35.3818

85.1372	120.6073	35.4701	84.9805	120.2532	35.2727	85.1692	120.6316	35.4623
85.1636	120.5564	35.3928	84.9692	120.2592	35.29	85.2801	120.5909	35.3108
85.1554	120.5268	35.3714	84.9727	120.2748	35.3021	85.2558	120.5753	35.3195
85.1779	120.5129	35.335	84.9663	120.2455	35.2792	85.2758	120.59	35.3143
85.0766	120.5207	35.4442	84.9701	120.2687	35.2987	85.255	120.5917	35.3368
85.0549	120.5225	35.4675	85.0376	120.3103	35.2727	85.2737	120.5441	35.2704
85.0826	120.5216	35.439	85.0567	120.3086	35.2519	85.2645	120.5103	35.2459
85.0645	120.5233	35.4589	85.1147	120.3112	35.1965	85.3043	120.4965	35.1922
85.1086	120.5251	35.4164	85.1381	120.3406	35.2026	85.3485	120.5389	35.1904
85.1398	120.5767	35.4369	85.1372	120.3545	35.2173	85.3537	120.5796	35.2259
85.1571	120.5848	35.4277	85.1294	120.3346	35.2052	85.3658	120.5935	35.2277
85.1666	120.5649	35.3983	85.0694	120.338	35.2686	85.3598	120.6645	35.3047
85.1485	120.5554	35.4069	85.0125	120.3337	35.3212	85.3979	120.6957	35.2978
85.1536	120.5251	35.3714	84.9675	120.293	35.3255	85.4358	120.7018	35.266
85.1173	120.5294	35.4121	84.9874	120.3354	35.348	85.4247	120.7147	35.29
85.1017	120.5389	35.4372	84.9753	120.3493	35.374	85.4134	120.7312	35.3177
85.0852	120.4965	35.4113	84.9831	120.3848	35.4017	85.3701	120.7138	35.3437
85.0852	120.4868	35.4016	85.0956	120.3718	35.2762	85.3381	120.7208	35.3827
85.0887	120.4991	35.4104	85.0956	120.422	35.3264	85.3407	120.6844	35.3437
85.0697	120.4861	35.4164	85.0746	120.4595	35.3849	85.3675	120.7615	35.3939
85.0246	120.5095	35.4849	85.1017	120.4515	35.3498	85.4238	120.7641	35.3402
85.0671	120.4861	35.419	85.0774	120.422	35.3446	85.4525	120.8225	35.3699
85.1337	120.5121	35.3783	85.0255	120.4307	35.4052	85.5052	120.8697	35.3645
85.0541	120.5476	35.4935	85.0272	120.4099	35.3827	85.5581	120.8983	35.3402
85.0792	120.5484	35.4693	84.9527	120.4411	35.4883	85.5252	120.9529	35.4277
85.0773	120.5327	35.4554	84.9857	120.4151	35.4294	85.487	120.984	35.497
85.0012	120.5528	35.5515	84.9657	120.4567	35.4909	85.4862	121.0325	35.5463
84.4152	120.282	35.8668	85.0059	120.4454	35.4395	85.5052	121.0585	35.5533
85.1207	120.1622	35.0415	85.0532	120.4541	35.4009	85.5113	121.0836	35.5723
85.0896	120.0548	34.9653	85.0887	120.4272	35.3385	85.5292	121.1255	35.5963
85.0956	120.0964	35.0008	85.0948	120.4636	35.3688	85.5728	121.139	35.5663
85.074	120.1241	35.0501	85.0852	120.4662	35.3809	85.6022	121.165	35.5628
85.093	120.1328	35.0398	85.1277	120.461	35.3333	85.6429	121.1771	35.5342
85.0913	120.1683	35.077	85.0818	120.4766	35.3948	85.6308	121.2005	35.5697
85.0923	120.2032	35.1109	85.0359	120.5493	35.5134	85.6282	121.1953	35.5671
85.0913	120.2116	35.1203	85.0385	120.5779	35.5394	85.5503	121.249	35.6988
85.1147	120.2332	35.1186	85.0826	120.6366	35.554	85.5468	121.2499	35.7031
85.1095	120.2722	35.1627	85.1918	120.642	35.4502	85.6226	121.2215	35.599
85.1216	120.2817	35.1601	85.261	120.6602	35.3991	85.6403	121.2724	35.6321
85.1389	120.2982	35.1593	85.216	120.6567	35.4407	85.649	121.3122	35.6632
85.1225	120.2982	35.1757	85.216	120.6117	35.3957	85.6784	121.3287	35.6503
85.0731	120.3268	35.2536	85.2151	120.6368	35.4216	85.7252	121.3434	35.6182
85.0826	120.3397	35.2572	85.2541	120.5926	35.3385	85.7235	121.3642	35.6407
85.0506	120.306	35.2554	85.2333	120.6099	35.3766	85.6854	121.3625	35.6771
85.0229	120.3354	35.3125	85.2861	120.5688	35.2827	85.6637	121.3417	35.678
85.0021	120.3216	35.3195	85.2342	120.6082	35.374	85.655	121.3607	35.7057
85.0177	120.2887	35.271	85.1926	120.5926	35.4	85.6772	121.3836	35.7064

85.7027	121.4343	35.7317	85.7166	124.5887	38.8721	85.1528	124.512	39.3592
85.7252	121.4733	35.7481	86.2254	124.5629	38.3375	85.2065	124.5319	39.3254
85.7659	121.4967	35.7308	85.6168	124.5088	38.8919	85.2065	124.5466	39.3402
85.8274	121.553	35.7256	86.1434	124.5105	38.3671	85.2006	124.5892	39.3886
85.8698	121.5538	35.684	85.9507	124.4623	38.5116	85.1285	124.5215	39.393
85.9027	121.6075	35.7048	85.7747	124.4061	38.6314	85.3058	124.5089	39.2031
85.9503	121.663	35.7126	86.1253	124.3989	38.2736	85.2752	124.4723	39.1971
85.9353	121.6752	35.7399	86.0923	124.3816	38.2893	85.0056	124.4609	39.4553
85.9642	121.6915	35.7273	85.884	124.3628	38.4787	84.9796	124.46	39.4804
86.0231	121.7643	35.7412	85.8973	124.3394	38.4421	85.1159	124.4357	39.3197
86.0595	121.7868	35.7273	85.8909	124.3314	38.4405	84.8497	124.4505	39.6008
86.0785	121.8595	35.781	85.1994	124.3462	39.1469	85.0298	124.4306	39.4008
86.0872	121.9098	35.8226	85.7844	124.3777	38.5933	85.0427	124.428	39.3853
86.1296	121.9236	35.794	86.1528	124.4264	38.2736	85.0606	124.4101	39.3495
86.1807	122.0033	35.8226	85.6317	124.4766	38.8449	85.035	124.422	39.387
86.2084	122.0284	35.8201	85.0697	124.5077	39.438	84.8462	124.4687	39.6225
86.1937	122.1185	35.9248	85.8607	124.4856	38.6249	85.0929	124.4944	39.4015
86.2742	122.0483	35.7741	85.2879	124.4652	39.1774	85.3391	124.5319	39.1928
86.2751	122.0474	35.7724	85.0116	124.4237	39.412	85.3835	124.5963	39.2129
86.2742	122.0855	35.8113	85.2138	124.3914	39.1775	85.408	124.6537	39.2457
86.2638	122.07	35.8061	85.9819	124.4288	38.4469	85.2198	124.6971	39.4773
86.2326	122.0881	35.8555	85.4153	124.4741	39.0588	85.0315	124.68	39.6484
86.237	122.0942	35.8572	85.2701	124.4927	39.2227	85.207	124.6528	39.4458
86.2471	122.135	35.8879	85.1277	124.531	39.4034	84.9813	124.6402	39.6588
86.3409	122.2631	35.9222	85.1216	124.5345	39.4129	85.1926	124.5855	39.393
86.4924	122.3756	35.8832	85.0614	124.4782	39.4168	85.3818	124.5587	39.1769
86.534	122.4891	35.9551	85.4111	124.4037	38.9926	85.1679	124.5055	39.3376
86.6145	122.5644	35.9499	85.3558	124.3669	39.011	84.9407	124.4553	39.5146
86.7107	122.6069	35.8962	84.8852	124.3743	39.4891	84.932	124.4245	39.4926
86.7626	122.651	35.8884	84.8722	124.4037	39.5315	84.9372	124.4774	39.5402
86.8033	122.7316	35.9282	84.8601	124.4427	39.5826	84.9735	124.4851	39.5116
86.8276	122.7587	35.9311	85.2553	124.464	39.2087	85.259	124.5821	39.3231
86.844	122.8138	35.9698	85.314	124.5126	39.1986	85.1164	124.641	39.5246
86.8536	122.8216	35.9681	85.3245	124.4936	39.169	85.1597	124.6618	39.5021
86.8839	122.8485	35.9646	85.2897	124.5143	39.2246	85.3808	124.663	39.2823
86.8943	122.8675	35.9733	85.3173	124.4933	39.176	85.3598	124.6376	39.2778
86.9202	122.8944	35.9741	85.9172	124.4951	38.5778	85.1337	124.6419	39.5082
85.8889	123.323	37.4342	85.1892	124.5208	39.3317	85.3143	124.5992	39.2848
85.1433	123.7473	38.6041	85.6424	124.4989	38.8565	85.2947	124.5923	39.2976
84.9152	123.8114	38.8962	85.4538	124.4875	39.0336	85.2845	124.5906	39.3061
86.255	124.3164	38.0614	84.9831	124.4566	39.4735	85.4957	124.6324	39.1367
86.1016	124.4655	38.3639	85.2177	124.4514	39.2336	86.1866	123.9163	37.7297
86.3077	124.5137	38.206	85.0255	124.4193	39.3938	82.6276	120.8818	38.2542
86.1583	124.5477	38.3894	85.4086	124.4179	39.0093	83.0303	121.1399	38.1096
86.3572	124.5813	38.2241	85.0497	124.3596	39.3098	83.1359	121.4543	38.3183
86.1639	124.6036	38.4397	85.2999	124.3931	39.0932	83.4633	121.5192	38.0559
86.299	124.6064	38.3074	85.3646	124.4305	39.066	83.6503	121.5841	37.9338

83.9465	121.6084	37.6619	81.2602	120.215	38.9548	78.4684	118.2164	39.748
83.9785	121.7218	37.7433	81.2005	120.1129	38.9124	78.4528	118.167	39.7143
84.2501	121.6488	37.3986	81.1537	120.0938	38.9401	78.5039	118.1549	39.651
84.1621	121.895	37.7329	81.1338	120.0427	38.9089	78.451	118.1359	39.6848
83.9222	122.1574	38.2352	81.0745	119.9909	38.9164	78.3445	118.0796	39.735
84.079	122.1609	38.0819	80.9944	119.976	38.9816	78.3402	118.0371	39.6969
84.4029	122.0509	37.6481	81.0134	119.9163	38.9028	78.2071	118.0027	39.7956
84.3275	122.1566	37.829	80.8896	119.8938	39.0042	78.1445	117.9687	39.8242
84.3344	122.1661	37.8316	80.7666	119.8609	39.0942	78.1124	117.9211	39.8086
84.2756	122.2206	37.9451	80.6948	119.8392	39.1444	78.1012	117.8856	39.7844
84.236	122.1588	37.9228	80.6255	119.7925	39.167	78.1575	117.8077	39.6502
84.0469	122.2717	38.2248	80.4904	119.7483	39.2579	78.0691	117.7505	39.6814
84.0426	122.3419	38.2993	80.3592	119.6702	39.3111	78.0181	117.7011	39.6831
83.8538	122.354	38.5002	80.2194	119.6652	39.4458	77.9531	117.6085	39.6554
83.7785	122.257	38.4785	80.2029	119.608	39.4051	77.9517	117.5349	39.5833
83.8244	122.1306	38.3062	80.0678	119.5673	39.4995	77.8648	117.515	39.6502
83.5897	122.1202	38.5305	80.0834	119.4885	39.4051	77.6994	117.5002	39.8009
83.5733	122.0431	38.4699	79.8808	119.4868	39.606	77.6492	117.4422	39.7931
83.5181	121.9897	38.4716	79.8089	119.4339	39.6251	77.5799	117.3816	39.8017
83.4875	121.8673	38.3798	79.8894	119.3898	39.5004	77.6145	117.327	39.7125
83.4901	121.8379	38.3478	79.7461	119.3619	39.6159	77.5227	117.3011	39.7783
83.407	121.8128	38.4058	79.6114	119.3673	39.7558	77.5193	117.2283	39.7091
83.1186	121.7452	38.6266	79.6106	119.3482	39.7376	77.4372	117.1843	39.7471
83.0234	121.6777	38.6543	79.5907	119.285	39.6943	77.4976	117.1227	39.6251
82.9922	121.6015	38.6093	79.4989	119.2296	39.7307	77.4543	117.1157	39.6614
82.8277	121.5183	38.6907	79.4634	119.1932	39.7298	77.2413	117.1374	39.8961
82.772	121.5078	38.7358	79.4296	119.1499	39.7203	77.2577	117.127	39.8693
82.9108	121.5192	38.6084	79.3716	119.0789	39.7073	77.1088	117.0672	39.9585
82.8043	121.4283	38.624	79.288	118.9646	39.6766	77.1633	117.0291	39.8658
82.625	121.378	38.753	79.3846	118.8494	39.4649	77.1374	116.9486	39.8112
82.5783	121.2889	38.7106	79.4166	118.7394	39.3228	76.9369	116.9668	40.0299
82.4025	121.2204	38.818	79.3291	118.6615	39.3324	76.9486	116.8863	39.9377
82.3323	121.139	38.8067	79.2027	118.626	39.4233	76.9183	116.8508	39.9325
82.2561	121.0377	38.7816	79.1161	118.6563	39.5402	76.875	116.8023	39.9273
82.0526	121.0031	38.9505	79.0416	118.6442	39.6025	76.8299	116.7538	39.9238
81.9245	120.959	39.0345	79.0529	118.5983	39.5454	76.7182	116.7087	39.9905
81.966	120.8862	38.9202	79.0157	118.5351	39.5194	76.6992	116.6429	39.9437
81.8768	120.8706	38.9938	78.7075	118.4757	39.7683	76.642	116.604	39.9619
81.8933	120.8039	38.9106	78.8892	118.3991	39.5099	76.6827	116.5416	39.8589
81.9262	120.7234	38.7972	78.6849	118.3974	39.7125	76.6347	116.4858	39.8511
81.8084	120.6385	38.8301	78.8814	118.3082	39.4268	76.545	116.4671	39.9221
81.6517	120.5969	38.9453	78.8537	118.3082	39.4545	76.5554	116.3849	39.8294
81.6439	120.5242	38.8803	78.8286	118.3047	39.4761	76.5987	116.3225	39.7238
81.4568	120.4798	39.023	78.684	118.2917	39.6077	76.481	116.3009	39.8199
81.4057	120.416	39.0102	78.4164	118.335	39.9186	76.4273	116.2437	39.8164
81.3694	120.3744	39.005	78.5234	118.2494	39.726	76.3199	116.2446	39.9247
81.2793	120.2904	39.0111	78.458	118.2233	39.7653	76.242	116.2264	39.9844

76.1784	116.1449	39.9665	74.3896	114.51	40.1204
76.1129	116.0645	39.9515	74.3836	114.4789	40.0953
76.1207	116.0367	39.916			
76.0861	116.0168	39.9307			
76.1744	115.9415	39.7671			
76.1476	115.8861	39.7385			
76.0393	115.8445	39.8052			
75.9943	115.8003	39.8061			
75.8807	115.7502	39.8696			
75.8237	115.7111	39.8875			
75.8116	115.6791	39.8675			
75.8436	115.628	39.7844			
75.7345	115.6072	39.8727			
75.7163	115.5622	39.8459			
75.5362	115.5622	40.026			
75.5578	115.4981	39.9403			
75.5248	115.4613	39.9365			
75.5345	115.3639	39.8294			
75.4487	115.3639	39.9152			
75.3881	115.324	39.9359			
75.3128	115.2297	39.9169			
75.2054	115.0054	39.8			
75.1448	114.9898	39.845			
75.027	114.9881	39.9611			
74.9804	114.9645	39.9841			
74.872	114.9984	40.1265			
74.8581	114.9681	40.11			
74.9049	114.9647	40.0598			
74.898	114.9551	40.0572			
74.8486	114.9283	40.0797			
74.8408	114.9153	40.0745			
74.8711	114.8634	39.9922			
74.7461	114.8544	40.1083			
74.7585	114.749	39.9905			
74.6823	114.7846	40.1022			
74.6261	114.7742	40.1481			
74.7256	114.7031	39.9775			
74.646	114.7006	40.0546			
74.6105	114.7031	40.0927			
74.4988	114.698	40.1992			
74.5187	114.6746	40.1559			
74.4836	114.6077	40.1241			
74.4667	114.5828	40.1161			
74.4936	114.5629	40.0693			
74.4122	114.5819	40.1698			
74.4702	114.5239	40.0537			
74.4234	114.5274	40.1039			

Table A.3: 18 Hour Test			80.138	119.9068	39.7688	79.8522	120.0566	40.2044
minimum	maximum	height	80.0548	119.9492	39.8944	79.8456	120.0393	40.1937
mmHg	mmHg	mmHg	80.0314	119.9241	39.8926	79.8955	120.0419	40.1464
80.519	119.6513	39.1323	80.015	119.9838	39.9689	79.9275	120.0626	40.1351
80.5935	119.6981	39.1046	80.1172	119.9968	39.8797	79.9396	120.054	40.1143
80.5882	119.6799	39.0917	80.1198	119.9812	39.8615	79.9085	120.1146	40.2061
80.6705	119.6608	38.9903	80.1717	119.9821	39.8104	79.9422	120.1311	40.1888
80.6627	119.6652	39.0024	80.0923	119.9935	39.9013	79.8998	120.157	40.2572
80.5926	119.66	39.0674	80.0574	120.0159	39.9585	79.8669	120.1873	40.3204
80.5129	119.653	39.1401	80.0825	120.0237	39.9411	79.8121	120.2191	40.4069
80.506	119.6236	39.1176	80.0652	120.0219	39.9567	79.7907	120.2765	40.4858
80.4921	119.679	39.1869	80.1007	120.0219	39.9212	79.7465	120.2722	40.5257
80.551	119.6626	39.1115	80.0851	120.0271	39.942	79.6885	120.3016	40.6131
80.4711	119.702	39.2309	80.0462	120.0678	40.0217	79.7665	120.325	40.5586
80.4255	119.7214	39.296	80.0747	120.0903	40.0156	79.718	120.3562	40.6382
80.3822	119.7353	39.3531	80.1081	120.0446	39.9365	79.6487	120.3501	40.7015
80.3943	119.75	39.3557	80.1362	120.0765	39.9403	79.6825	120.3614	40.6789
80.4159	119.7214	39.3055	80.1336	120.0124	39.8788	79.6501	120.3353	40.6853
80.4194	119.7301	39.3107	80.0877	120.0609	39.9732	79.7855	120.2956	40.5101
80.3588	119.7916	39.4328	80.0574	120.0237	39.9663	79.8305	120.2861	40.4555
80.383	119.7647	39.3817	80.0747	120.0592	39.9844	79.8617	120.2713	40.4096
80.4948	119.7302	39.2353	80.1683	120.0297	39.8615	79.8678	120.3103	40.4425
80.4332	119.7647	39.3315	80.1665	120.0297	39.8632	79.9327	120.2904	40.3577
80.4324	119.7864	39.354	80.19	120.0103	39.8202	79.8643	120.3597	40.4954
80.3986	119.7925	39.3938	80.2557	119.9397	39.6839	79.8357	120.3856	40.5499
80.3951	119.7977	39.4025	80.1916	119.9682	39.7766	79.8104	120.3618	40.5514
80.2566	119.7916	39.535	80.1267	120.0107	39.884	79.8937	120.3389	40.4451
80.2436	119.8297	39.5861	80.0704	120.0583	39.9879	79.8418	120.3475	40.5058
80.3735	119.7873	39.4138	80.0366	120.0566	40.0199	79.9353	120.3129	40.3776
80.3441	119.7595	39.4155	80.0843	120.0756	39.9914	79.9613	120.2878	40.3265
80.3865	119.8401	39.4536	80.0488	120.1007	40.052	79.9422	120.3164	40.3741
80.4064	119.7492	39.3428	79.9812	120.0644	40.0832	80.0063	120.3016	40.2953
80.2912	119.8721	39.5809	80.0059	120.0261	40.0202	80.0167	120.2376	40.2208
80.2237	119.8964	39.6727	80.0609	119.9717	39.9108	79.9838	120.2402	40.2564
80.1864	119.8765	39.69	80.0366	119.9899	39.9533	79.9795	120.2728	40.2933
80.1466	119.8721	39.7255	80.0903	119.9968	39.9065	80.0107	120.2661	40.2555
80.1683	119.8903	39.7221	80.06	120.0089	39.9489	79.9353	120.254	40.3187
80.157	119.899	39.742	80.0418	120.0107	39.9689	79.9422	120.2462	40.304
80.1451	119.8482	39.7031	80.1405	120.0219	39.8814	79.9717	120.2454	40.2737
80.1466	119.8756	39.729	80.1769	119.9778	39.8009	79.9691	120.2376	40.2685
80.1362	119.8955	39.7593	80.102	119.9645	39.8625	79.9786	120.2722	40.2936
80.0765	119.8877	39.8112	80.1258	120.0003	39.8745	79.9466	120.2739	40.3274
80.0522	119.9258	39.8736	80.0617	120.0038	39.942	79.9126	120.308	40.3955
80.0981	119.9657	39.8675	79.9907	120.0609	40.0702	79.8937	120.3094	40.4157
80.0808	119.9769	39.8961	79.9163	119.9934	40.0771	79.9024	120.3129	40.4105
80.0219	119.996	39.974	79.9604	119.9795	40.0191	79.9215	120.2748	40.3533
80.079	119.9997	39.9207	79.8513	120.0323	40.181	79.957	120.2635	40.3066

79.8929	120.2748	40.3819	79.5508	120.894	41.3431	78.9845	121.3564	42.3719
79.9379	120.3077	40.3698	79.485	120.9096	41.4245	79.0183	121.3157	42.2974
79.9786	120.2549	40.2763	79.3941	120.9407	41.5467	78.9914	121.2819	42.2905
79.9777	120.3239	40.3461	79.4365	120.9087	41.4722	78.9914	121.2646	42.2732
79.9396	120.3423	40.4027	79.4824	120.9096	41.4271	78.9758	121.2187	42.2429
79.9145	120.383	40.4685	79.3717	120.9837	41.612	78.9594	121.2222	42.2628
79.8548	120.3744	40.5196	79.2677	121.0724	41.8047	78.9057	121.2334	42.3278
79.8738	120.3856	40.5118	79.3248	121.0784	41.7536	78.8528	121.2418	42.3889
79.879	120.3744	40.4954	79.3776	121.0412	41.6636	78.891	121.2326	42.3416
79.8721	120.3683	40.4962	79.3958	121.0498	41.654	78.9091	121.2239	42.3148
79.8392	120.3839	40.5447	79.4486	121.0325	41.5839	78.9057	121.2161	42.3104
79.8139	120.3609	40.547	79.3811	121.1364	41.7553	78.9351	121.1979	42.2628
79.7985	120.3545	40.556	79.3525	121.1754	41.8229	78.9836	121.1546	42.171
79.7119	120.3744	40.6625	79.3602	121.1739	41.8137	78.9784	121.2014	42.223
79.7214	120.3536	40.6322	79.4027	121.1702	41.7675	78.9091	121.1832	42.2741
79.6747	120.4168	40.7422	79.3517	121.1823	41.8307	78.9665	121.1114	42.1449
79.6253	120.3839	40.7586	79.4573	121.1382	41.6809	78.9031	121.1425	42.2394
79.6599	120.4281	40.7681	79.459	121.146	41.6869	78.8676	121.1719	42.3044
79.377	124.2254	44.8484	79.3672	121.1607	41.7935	78.8979	121.1763	42.2784
79.9258	120.3732	40.4474	79.3127	121.1719	41.8593	78.8944	121.217	42.3226
79.8548	120.4835	40.6287	79.3742	121.1728	41.7986	78.8243	121.2265	42.4022
79.7699	120.5484	40.7785	79.4043	121.1801	41.7758	78.8364	121.2759	42.4395
79.7916	120.5441	40.7526	79.4867	121.1771	41.6904	78.9143	121.2508	42.3364
79.7742	120.577	40.8028	79.4495	121.1875	41.738	78.8828	121.255	42.3722
79.7829	120.6151	40.8322	79.3491	121.2456	41.8965	78.8433	121.2871	42.4438
79.7786	120.6489	40.8703	79.3439	121.23	41.8861	78.8615	121.3399	42.4784
79.7292	120.6775	40.9483	79.3265	121.2118	41.8852	78.7585	121.2837	42.5252
79.7531	120.7388	40.9857	79.2616	121.1642	41.9026	78.7827	121.2871	42.5044
79.7335	120.7927	41.0591	79.2295	121.1685	41.9389	78.8009	121.2992	42.4984
79.6712	120.8178	41.1466	79.2492	121.1519	41.9027	78.8251	121.2845	42.4594
79.7387	120.7918	41.053	79.2157	121.1728	41.9571	78.8243	121.2715	42.4473
79.7119	120.8022	41.0903	79.1447	121.146	42.0013	78.8431	121.2559	42.4127
79.7067	120.8455	41.1388	79.2218	121.1901	41.9684	78.8355	121.314	42.4784
79.7223	120.8671	41.1448	79.194	121.1702	41.9762	78.8122	121.2698	42.4577
79.7292	120.8126	41.0834	79.0962	121.2334	42.1372	78.7195	121.3192	42.5997
79.798	120.789	40.991	79.0901	121.2828	42.1927	78.729	121.3763	42.6473
79.7595	120.8385	41.079	79.0035	121.301	42.2974	78.684	121.3884	42.7045
79.6998	120.8394	41.1396	79.0132	121.2682	42.255	78.755	121.411	42.656
79.7725	120.823	41.0504	78.9784	121.2811	42.3026	78.6961	121.4378	42.7417
79.7283	120.8082	41.0799	78.9576	121.2871	42.3295	78.6606	121.4491	42.7885
79.7309	120.8619	41.131	78.9628	121.3088	42.3459	78.6564	121.4752	42.8188
79.718	120.881	41.163	78.9663	121.333	42.3667	78.5983	121.4283	42.83
79.6071	120.8611	41.254	78.9931	121.2906	42.2974	78.6095	121.4664	42.8569
79.5907	120.8663	41.2756	78.9888	121.3088	42.32	78.5446	121.4594	42.9149
79.5461	120.8269	41.2808	78.9559	121.3469	42.391	78.6112	121.4352	42.824
79.5318	120.8498	41.318	79.0148	121.359	42.3442	78.6545	121.4499	42.7954
79.5552	120.8671	41.312	78.9726	121.3607	42.3881	78.6035	121.4569	42.8534

78.5991	121.4941	42.895	78.3644	121.4421	43.0777	78.0769	121.7539	43.6769
78.6493	121.462	42.8127	78.3731	121.4084	43.0353	78.0631	121.7348	43.6717
78.7403	121.4491	42.7088	78.4554	121.398	42.9426	78.1298	121.7244	43.5947
78.6996	121.482	42.7824	78.5119	121.3607	42.8488	78.2337	121.6171	43.3834
78.6606	121.4612	42.8006	78.4891	121.3798	42.8906	78.2475	121.5582	43.3106
78.5844	121.514	42.9296	78.3774	121.4439	43.0664	78.2151	121.632	43.417
78.6242	121.5036	42.8794	78.4917	121.3928	42.901	78.2545	121.6647	43.4102
78.5697	121.5001	42.9305	78.4268	121.4551	43.0283	78.2371	121.6638	43.4267
78.5879	121.5391	42.9513	78.4606	121.4196	42.959	78.1237	121.7114	43.5877
78.5983	121.4964	42.8981	78.4649	121.391	42.9261	78.0691	121.7807	43.7116
78.5783	121.5409	42.9625	78.3974	121.443	43.0456	78.0553	121.8197	43.7644
78.6147	121.5677	42.953	78.3261	121.5184	43.1923	77.9808	121.8249	43.8441
78.5983	121.4958	42.8976	78.3939	121.4854	43.0915	78.0181	121.8128	43.7947
78.5948	121.4768	42.882	78.3809	121.5183	43.1374	78.0635	121.7668	43.7032
78.5558	121.4681	42.9123	78.3999	121.5279	43.1279	78.0605	121.7565	43.696
78.5931	121.4032	42.8101	78.4432	121.5642	43.121	78.0562	121.8067	43.7505
78.6312	121.3928	42.7616	78.387	121.5859	43.1989	78.0778	121.7773	43.6995
78.5128	121.4532	42.9404	78.4095	121.5521	43.1426	78.135	121.7357	43.6007
78.5792	121.4248	42.8456	78.3168	121.5997	43.2829	78.1393	121.7028	43.5635
78.5636	121.4343	42.8707	78.2767	121.632	43.3553	78.141	121.6404	43.4994
78.5723	121.4569	42.8846	78.2155	121.6751	43.4596	78.1029	121.7262	43.6232
78.5454	121.4941	42.9487	78.1609	121.6803	43.5193	78.0521	121.7175	43.6654
78.4943	121.5244	43.0301	78.2415	121.6543	43.4128	78.0276	121.747	43.7194
78.5333	121.5469	43.0136	78.2233	121.7045	43.4812	78.0657	121.7002	43.6345
78.5307	121.5036	42.9729	78.2068	121.7097	43.5029	78.1142	121.6898	43.5756
78.4873	121.4655	42.9783	78.2259	121.6855	43.4596	78.1124	121.682	43.5696
78.4926	121.4447	42.9521	78.219	121.6811	43.4622	78.096	121.6586	43.5626
78.49	121.4594	42.9694	78.2318	121.6857	43.4539	78.0969	121.6266	43.5297
78.3939	121.5088	43.1149	78.2086	121.7028	43.4942	78.0674	121.669	43.6016
78.406	121.5123	43.1063	78.1774	121.7019	43.5245	78.0213	121.6664	43.6451
78.4536	121.4854	43.0318	78.1462	121.6855	43.5392	78.0821	121.6612	43.5791
78.535	121.4049	42.8699	78.1817	121.6794	43.4977	78.0726	121.6924	43.6198
78.5775	121.4187	42.8413	78.2172	121.6508	43.4336	78.0804	121.6915	43.6111
78.4608	121.4453	42.9844	78.2207	121.6751	43.4544	78.083	121.6673	43.5843
78.4181	121.4664	43.0482	78.2744	121.6889	43.4145	78.1055	121.6482	43.5427
78.3393	121.4465	43.1071	78.1851	121.7457	43.5605	78.0856	121.6725	43.5869
78.3307	121.462	43.1314	78.1601	121.8015	43.6414	78.1289	121.6604	43.5315
78.3324	121.4283	43.0959	78.1765	121.8136	43.6371	78.0997	121.6549	43.5553
78.3108	121.4084	43.0976	78.135	121.818	43.683	78.0951	121.6439	43.5488
78.3064	121.4291	43.1227	78.0891	121.8509	43.7618	78.0821	121.6396	43.5574
78.3575	121.3858	43.0283	78.0077	121.8864	43.8787	78.0172	121.6933	43.6761
78.334	121.3977	43.0637	78.0562	121.876	43.8198	78.0224	121.7599	43.7376
78.3653	121.3685	43.0032	78.1661	121.85	43.6839	78.0198	121.7478	43.728
78.4415	121.3417	42.9002	78.075	121.8434	43.7684	78.0588	121.7279	43.6691
78.4147	121.3555	42.9409	78.1047	121.8318	43.7272	78.0319	121.7513	43.7194
78.3982	121.3893	42.9911	78.0813	121.8483	43.767	78.0276	121.8284	43.8008
78.3757	121.4006	43.0249	78.0657	121.8439	43.7783	78.0468	121.8566	43.8098

78.1185	121.8387	43.7202	77.9955	121.7521	43.7566	78.2181	123.2719	45.0538
78.1471	121.8309	43.6839	77.964	121.7967	43.8327	78.1912	123.2806	45.0893
78.1194	121.8292	43.7098	77.9903	121.8361	43.8458	78.1722	123.2936	45.1214
78.044	121.8699	43.8259	78.0856	121.792	43.7064	78.2027	123.2943	45.0915
78.0847	121.8448	43.7601	78.1523	121.7695	43.6172	78.1471	123.297	45.15
78.1332	121.8284	43.6951	78.2094	121.7314	43.5219	78.1436	123.2988	45.1551
78.1384	121.8015	43.6631	78.1583	121.747	43.5886	78.1869	123.22	45.033
78.075	121.8346	43.7596	78.1531	121.7625	43.6094	78.2571	123.1602	44.9031
78.0562	121.8708	43.8146	78.0865	121.8327	43.7462	78.3463	123.1221	44.7759
78.0328	121.8881	43.8553	78.0398	121.7871	43.7473	78.3194	123.1221	44.8027
77.9358	121.9808	44.045	78.0033	121.8162	43.8129	78.2761	123.1628	44.8867
77.986	121.9929	44.0069	78.012	121.8543	43.8423	78.2644	123.1234	44.859
78.0657	122.0024	43.9367	77.9531	121.9011	43.948	78.3255	123.0745	44.749
78.1358	121.9349	43.799	77.9488	121.9141	43.9653	78.3896	123.0442	44.6546
78.083	121.9989	43.9159	77.9401	121.9305	43.9904	78.3922	123.0728	44.6806
78.0653	121.9483	43.8829	77.9652	121.882	43.9168	78.3878	123.0771	44.6893
78.0943	121.9046	43.8103	78.0059	121.9201	43.9142	78.3774	123.0381	44.6607
78.0743	121.889	43.8146	78.0477	121.8707	43.823	78.3688	123.0217	44.6529
78.0388	121.8794	43.8406	78.0657	121.8206	43.7549	78.3523	123.0632	44.7109
78.0778	121.8881	43.8103	78.0553	121.8509	43.7956	78.2767	123.0423	44.7656
78.0631	121.8838	43.8207	78.0111	121.8994	43.8882	78.232	123.0217	44.7897
78.038	121.8898	43.8519	77.9834	121.9184	43.935	78.3315	122.9965	44.665
78.0129	121.9098	43.8969	77.9791	121.9262	43.9471	78.3913	122.9463	44.555
78.0424	121.854	43.8116	77.9349	121.9531	44.0181	78.4069	122.9506	44.5438
78.0025	121.8898	43.8874	77.8882	122.0258	44.1376	78.3168	122.9749	44.6581
77.999	121.8803	43.8813	77.9041	121.9879	44.0838	78.2536	122.974	44.7204
78.0016	121.9002	43.8986	77.9661	121.9695	44.0034	78.2804	123.0043	44.7239
78.0562	121.8569	43.8008	78.0475	121.9427	43.8952	78.2415	123.0177	44.7762
78.0284	121.8136	43.7852	77.9869	121.9712	43.9844	78.232	123.052	44.82
78.0544	121.8327	43.7783	77.941	122.0215	44.0805	78.2155	123.0442	44.8287
78.1012	121.8422	43.741	77.9384	122.0232	44.0848	78.1722	123.0494	44.8772
78.1675	121.7871	43.6196	77.9098	121.9773	44.0675	78.1774	123.0407	44.8633
78.1263	121.8032	43.6769	77.9237	121.9756	44.0519	78.1895	123.0875	44.898
78.096	121.8335	43.7376	77.8856	122.0346	44.149	78.2155	123.0676	44.8521
78.0336	121.8309	43.7973	77.8362	122.0821	44.2459	78.2986	123.0719	44.7733
78.0466	121.7946	43.7479	77.8674	122.102	44.2346	78.334	123.0749	44.7409
78.044	121.8145	43.7705	77.8916	122.1262	44.2346	78.2995	123.1186	44.8192
78.1176	121.7703	43.6527	77.8501	122.1297	44.2797	78.2657	123.1697	44.904
78.1038	121.7781	43.6743	77.8431	122.0812	44.2381	78.2172	123.1775	44.9603
78.0953	121.7571	43.6618	77.8518	122.0786	44.2268	78.2233	123.1862	44.9629
78.1246	121.7599	43.6354	77.8553	122.057	44.2017	78.2086	123.2243	45.0157
78.115	121.7556	43.6406	77.838	122.0478	44.2098	78.1817	123.2278	45.046
78.1627	121.74	43.5773	77.9644	122.5367	44.5723	78.1592	123.239	45.0798
78.1523	121.7132	43.5609	78.2285	123.2763	45.0478	78.1437	123.2379	45.0942
78.1722	121.714	43.5418	78.232	123.2823	45.0504	78.0536	123.2737	45.2201
78.1298	121.7045	43.5748	78.2519	123.323	45.0711	78.0449	123.2875	45.2426
78.1263	121.7028	43.5765	78.2094	123.317	45.1075	78.1549	123.2139	45.059

78.3038	123.1464	44.8425	78.2519	123.4096	45.1577	78.0345	123.7083	45.6738
78.322	123.1282	44.8062	78.1809	123.3975	45.2166	78.0588	123.7534	45.6946
78.2285	123.1914	44.9629	78.193	123.3481	45.1551	77.9981	123.7543	45.7561
78.2086	123.1741	44.9655	78.2155	123.3299	45.1144	77.9843	123.7742	45.7899
78.2068	123.1836	44.9768	78.2068	123.3949	45.1881	78.0085	123.7785	45.77
78.1737	123.1613	44.9876	78.2415	123.3706	45.1292	77.9826	123.7248	45.7423
78.2034	123.1827	44.9794	78.2242	123.349	45.1248	77.9877	123.6937	45.7059
78.1679	123.1464	44.9785	78.2195	123.4035	45.184	78.0051	123.7413	45.7362
78.1713	123.1498	44.9785	78.2155	123.3603	45.1448	78.0068	123.7014	45.6946
78.1038	123.1819	45.0781	78.2268	123.3646	45.1378	78.0662	123.6863	45.6201
78.1124	123.2018	45.0893	78.1774	123.4036	45.2262	78.0536	123.7136	45.66
78.1462	123.1767	45.0304	78.1402	123.3888	45.2487	78.0293	123.7309	45.7016
78.1488	123.181	45.0322	78.1073	123.3984	45.2911	77.9843	123.711	45.7267
78.1543	123.2176	45.0634	78.0622	123.452	45.3898	77.9609	123.7162	45.7553
78.1376	123.1923	45.0547	78.0423	123.4746	45.4323	78.025	123.717	45.692
78.1869	123.1931	45.0062	78.1041	123.4396	45.3356	77.9999	123.7023	45.7024
78.1635	123.1975	45.0339	78.1116	123.4261	45.3145	77.9756	123.7101	45.7345
78.1653	123.233	45.0677	78.1289	123.4417	45.3128	78.0169	123.6819	45.665
78.1913	123.2399	45.0486	78.1047	123.4694	45.3647	78.0068	123.6391	45.6323
78.1506	123.3031	45.1525	78.1168	123.4754	45.3587	77.9479	123.6521	45.7042
78.1402	123.3048	45.1647	78.0969	123.4832	45.3864	77.98	123.6123	45.6323
78.1904	123.3154	45.125	78.0795	123.5092	45.4297	78.031	123.5932	45.5622
78.1921	123.3144	45.1222	78.0761	123.465	45.389	78.0267	123.5733	45.5466
78.1869	123.2797	45.0928	78.1719	123.4951	45.3232	77.999	123.5664	45.5673
78.2337	123.2979	45.0642	78.1272	123.4737	45.3465	77.9419	123.6486	45.7068
78.2242	123.2944	45.0703	78.1073	123.5239	45.4167	77.9587	123.5955	45.6368
78.2666	123.2988	45.0322	78.1757	123.5213	45.3457	78.0068	123.5724	45.5656
78.1436	123.4079	45.2643	78.2077	123.5109	45.3032	78.0856	123.5499	45.4643
78.0674	123.4443	45.3768	78.1627	123.5031	45.3405	78.0492	123.5733	45.5241
78.1182	123.4238	45.3056	78.1081	123.5222	45.4141	78.0085	123.6287	45.6202
78.0432	123.4564	45.4132	78.025	123.5871	45.5622	77.9981	123.6607	45.6626
78.0865	123.4295	45.3431	78.0336	123.6264	45.5928	77.96	123.6512	45.6912
77.9851	123.5014	45.5163	78.0198	123.6045	45.5847	77.8665	123.7162	45.8497
78.0051	123.4945	45.4894	78.0388	123.5958	45.557	77.8812	123.6907	45.8095
78.0172	123.5127	45.4955	78.0423	123.6166	45.5743	77.8726	123.7499	45.8774
78.0666	123.4884	45.4219	78.0129	123.659	45.6462	77.8951	123.7283	45.8332
78.1471	123.4572	45.3102	77.967	123.6564	45.6895	77.8916	123.7352	45.8436
78.1516	123.4819	45.3303	77.9834	123.6599	45.6765	77.9289	123.7023	45.7735
78.1956	123.5213	45.3257	77.9938	123.6486	45.6548	77.883	123.7032	45.8202
78.1549	123.517	45.3621	77.9385	123.6678	45.7293	77.8232	123.7785	45.9553
78.1791	123.4884	45.3093	77.9202	123.7014	45.7812	77.8449	123.7785	45.9337
78.1835	123.4893	45.3058	77.9193	123.7118	45.7925	77.7755	123.829	46.0535
78.1047	123.504	45.3994	77.9713	123.6746	45.7033	77.8284	123.8296	46.0012
78.0336	123.4867	45.453	77.999	123.6911	45.692	77.8535	123.8261	45.9726
78.1488	123.485	45.3361	78.0362	123.6677	45.6314	77.8752	123.7863	45.9111
78.2388	123.3991	45.1603	78.0475	123.685	45.6375	77.8986	123.8088	45.9103
78.2822	123.3629	45.0807	78.0276	123.7118	45.6843	77.883	123.8218	45.9389

77.9029	123.8028	45.8999	77.6344	123.6988	46.0644	77.2395	123.8357	46.5961
77.8977	123.853	45.9553	77.6284	123.7006	46.0722	77.3409	123.8513	46.5104
77.8636	123.8431	45.9795	77.6673	123.6486	45.9813	77.2872	123.866	46.5788
77.7721	123.8616	46.0895	77.65	123.7283	46.0783	77.2751	123.8521	46.5771
77.8258	123.8417	46.0159	77.5976	123.6537	46.0561	77.295	123.8729	46.5779
77.8665	123.8911	46.0246	77.4933	123.6772	46.1839	77.2803	123.8452	46.5649
77.805	123.8842	46.0791	77.5755	123.6668	46.0913	77.2425	123.8721	46.6296
77.8223	123.8859	46.0635	77.5348	123.6365	46.1017	77.2066	123.9041	46.6974
77.779	123.9335	46.1545	77.5097	123.5577	46.048	77.1971	123.8686	46.6715
77.8535	123.9396	46.0861	77.521	123.614	46.093	77.2569	123.8599	46.603
77.8116	123.9814	46.1698	77.4595	123.6711	46.2116	77.2309	123.8573	46.6264
77.8596	123.9656	46.106	77.4223	123.6391	46.2168	77.2603	123.8192	46.5589
77.844	123.9682	46.1242	77.4474	123.6564	46.209	77.2127	123.9058	46.6931
77.8752	123.9569	46.0817	77.4319	123.6739	46.242	77.1642	123.9067	46.7425
77.8223	124.0071	46.1848	77.3729	123.6815	46.3086	77.1245	123.9541	46.8296
77.7176	124.0426	46.3251	77.4136	123.6521	46.2385	77.1027	123.9647	46.862
77.7236	124.0383	46.3147	77.3634	123.6677	46.3043	77.1599	123.9223	46.7624
77.7245	124.0219	46.2974	77.34	123.6729	46.3329	77.056	124.0565	47.0005
77.7262	124.0097	46.2835	77.4231	123.6304	46.2073	77.0741	124.0859	47.0118
77.6856	124.0149	46.3292	77.3045	123.6694	46.3649	76.9953	124.0617	47.0663
77.6639	123.9976	46.3337	77.3002	123.6694	46.3692	76.9936	124.1033	47.1096
77.6734	123.9941	46.3207	77.3588	123.6616	46.3028	76.9191	124.1197	47.2006
77.7028	123.9855	46.2826	77.3443	123.6928	46.3485	76.9685	124.1041	47.1356
77.6925	123.9863	46.2939	77.3062	123.7421	46.4359	76.9783	124.1329	47.1546
77.7531	123.9543	46.2012	77.3305	123.7924	46.4619	77.0499	124.0807	47.0308
77.7617	123.9578	46.196	77.3989	123.7344	46.3355	77.0542	124.0747	47.0204
77.8223	123.9292	46.1068	77.4024	123.7196	46.3173	77.017	124.0816	47.0646
77.7799	123.9285	46.1486	77.4015	123.7188	46.3173	76.9512	124.1232	47.172
77.721	123.9205	46.1995	77.4257	123.6902	46.2645	76.9512	124.1232	47.172
77.7002	123.9205	46.2203	77.439	123.703	46.264	77.0525	124.0643	47.0118
77.7513	123.8738	46.1224	77.4335	123.6443	46.2108	77.043	124.0392	46.9962
77.8189	123.8296	46.0107	77.4465	123.6746	46.2281	76.9836	124.0827	47.0991
77.7167	123.8599	46.1432	77.4751	123.7387	46.2636	76.972	124.0426	47.0707
77.7401	123.8374	46.0973	77.495	123.717	46.222	76.985	124.0565	47.0715
77.6803	123.8547	46.1744	77.5054	123.6997	46.1943	76.9806	124.0322	47.0516
77.6909	123.755	46.0641	77.4829	123.6945	46.2116	76.9503	124.1085	47.1581
77.6968	123.8019	46.1051	77.5071	123.6841	46.177	76.952	124.0418	47.0897
77.6942	123.8106	46.1164	77.5306	123.7127	46.1821	76.9036	124.0859	47.1824
77.6318	123.724	46.0921	77.4647	123.7395	46.2748	76.9235	124.0582	47.1348
77.6734	123.6729	45.9995	77.4864	123.7482	46.2619	77.0443	124.0113	46.967
77.6466	123.6703	46.0237	77.411	123.808	46.3969	77.1452	123.9266	46.7814
77.6673	123.6581	45.9908	77.4682	123.808	46.3398	77.2465	123.9145	46.668
77.6743	123.704	46.0298	77.4292	123.8166	46.3874	77.2811	123.885	46.6039
77.5843	123.6458	46.0614	77.3573	123.8192	46.4619	77.4101	123.8868	46.4766
77.5998	123.737	46.1372	77.321	123.7075	46.3866	77.54	123.6841	46.1441
77.5721	123.659	46.0869	77.2293	123.8413	46.612	77.4171	123.7621	46.345
77.5877	123.7318	46.1441	77.1876	123.8426	46.655	77.1729	123.7231	46.5502

77.2699	123.8545	46.5847	77.0412	124.0643	47.023	77.2664	123.9101	46.6437
77.3452	123.8755	46.5303	77.0664	124.1067	47.0404	77.301	123.9006	46.5996
77.3738	123.8634	46.4896	77.0972	124.0245	46.9273	77.2465	123.9543	46.7078
77.3417	123.84	46.4983	77.1408	124.0201	46.8793	77.1504	124.0089	46.8585
77.3677	123.8989	46.5312	77.133	124.0227	46.8897	77.1439	123.9708	46.8269
77.3132	123.8166	46.5035	77.1322	123.9967	46.8646	77.2309	123.9682	46.7373
77.3088	123.9041	46.5953	77.1599	123.9898	46.8299	77.3591	123.9517	46.5927
77.3036	123.8383	46.5346	77.0975	123.9898	46.8923	77.3668	123.9205	46.5537
77.2884	123.8845	46.5961	77.0949	124.0279	46.933	77.3954	123.9517	46.5563
77.2439	123.8868	46.6429	77.0811	124.0877	47.0066	77.3729	123.9257	46.5528
77.269	123.9041	46.6351	77.0813	124.0686	46.9872	77.2136	124.0366	46.823
77.3071	123.8703	46.5632	77.1123	124.1587	47.0464	77.1893	124.0167	46.8273
77.3097	123.8885	46.5788	77.0837	124.1033	47.0196	77.1606	124.0237	46.863
77.3642	123.8625	46.4983	77.1123	124.131	47.0187	77.1079	124.0634	46.9555
77.2924	123.7863	46.4939	77.0741	124.0712	46.9971	77.0603	124.0729	47.0127
77.3227	123.8469	46.5242	77.0915	124.1067	47.0153	77.0274	124.0548	47.0274
77.3826	123.8607	46.4781	77.0828	124.1517	47.0689	76.9772	124.0591	47.0819
77.3391	123.7993	46.4602	77.1209	124.1344	47.0135	76.9261	124.1033	47.1772
77.2811	123.795	46.5139	77.1474	124.0686	46.9212	76.9313	124.0859	47.1547
77.3409	123.8071	46.4662	77.1564	124.0123	46.8559	76.8654	124.0219	47.1564
77.3885	123.7404	46.3519	77.0741	123.9301	46.8559	76.8699	124.0809	47.211
77.4275	123.633	46.2056	77.1348	123.924	46.7892	76.8291	124.1041	47.275
77.2967	123.7292	46.4325	77.1573	123.8781	46.7208	76.8066	124.1552	47.3486
77.2569	123.7292	46.4723	77.1278	123.8816	46.7537	76.7866	124.2028	47.4162
77.2906	123.653	46.3623	77.1443	123.8547	46.7104	76.778	124.1812	47.4032
77.3227	123.6361	46.3134	77.2023	123.8946	46.6922	76.7485	124.1396	47.3911
77.2681	123.6356	46.3675	77.1547	123.9231	46.7684	76.8057	124.1067	47.301
77.2655	123.7153	46.4498	77.1967	123.9497	46.7529	76.8949	124.124	47.2291
77.3062	123.7413	46.4351	77.146	123.9344	46.7884	76.8862	124.0937	47.2075
77.3201	123.8062	46.4861	77.2352	123.9673	46.7321	76.9968	124.0633	47.0665
77.2733	123.8296	46.5563	77.2828	123.9482	46.6654	77.0014	124.0903	47.0889
77.2984	123.8426	46.5442	77.2915	123.872	46.5805	77.0109	124.0097	46.9988
77.321	123.7802	46.4593	77.2395	123.8859	46.6463	76.9417	124.0608	47.1192
77.3069	123.8607	46.5538	77.2742	123.8842	46.61	76.9858	124.1214	47.1356
77.2629	123.7984	46.5355	77.3149	123.8521	46.5372	77.0395	124.0643	47.0248
77.2889	123.8019	46.513	77.3853	123.8184	46.4332	76.9988	124.0288	47.03
77.2404	123.8469	46.6065	77.3642	123.7976	46.4333	77.0473	124.0634	47.0161
77.2967	123.8504	46.5537	77.3591	123.8296	46.4706	77.0214	124.0827	47.0612
77.3642	123.7932	46.429	77.3781	123.7932	46.4151	76.959	124.0885	47.1296
77.3729	123.9335	46.5606	77.4136	123.7958	46.3822	76.92	124.1266	47.2066
77.3339	124.0011	46.6671	77.4145	123.7872	46.3727	76.8836	124.1509	47.2672
77.3157	123.9479	46.6322	77.4093	123.7794	46.3701	76.8377	124.2124	47.3746
77.2777	124.0011	46.7234	77.4249	123.7629	46.3381	76.8906	124.1162	47.2257
77.185	124.0461	46.8611	77.4487	123.799	46.3503	76.8646	124.1492	47.2846
77.1174	124.1266	47.0092	77.4214	123.827	46.4056	76.8932	124.1128	47.2196
77.1382	124.1024	46.9642	77.3816	123.8192	46.4376	76.9589	124.1681	47.2092
77.1876	124.1509	46.9633	77.3201	123.8608	46.5407	76.8802	124.1881	47.308

76.8732	124.2609	47.3876
76.8325	124.2098	47.3772
76.8966	124.1821	47.2854
76.8888	124.2028	47.314
76.8525	124.1743	47.3218
76.8308	124.1708	47.34
76.8197	124.1576	47.3378
76.8317	124.1474	47.3157
76.9027	124.1569	47.2543
76.907	124.176	47.269
76.9486	124.1422	47.1936
76.9417	124.1604	47.2188
76.9927	124.1855	47.1928
76.9598	124.1708	47.211
77.0012	124.1391	47.1379
77.0101	124.1595	47.1495
76.9728	124.1266	47.1538
76.9624	124.1838	47.2214
76.933	124.1214	47.1884
76.9425	124.1258	47.1832
76.9417	124.1379	47.1962
77.0057	124.1085	47.1027
76.9809	124.0545	47.0736
77.062	124.0435	46.9815
77.03	124.0721	47.0421
77.153	124.0331	46.8802
77.1642	124.079	46.9148
77.1252	124.0426	46.9174
77.1105	124.0461	46.9356
77.0958	124.0963	47.0005
77.1443	124.1024	46.9581
77.1351	124.0985	46.9635
77.1157	124.1059	46.9901
77.0231	124.1353	47.1122
76.9997	124.1457	47.146
77.0118	124.1569	47.1451
76.9919	124.1621	47.1703
76.978	124.1916	47.2136
76.9798	124.1673	47.1876
76.9712	124.1893	47.218
76.9633	124.1751	47.2118
77.0404	124.1535	47.1131
77.0343	124.0989	47.0646
77.0031	124.2409	47.2378
76.9624	124.2583	47.2958
76.9165	124.2669	47.3504
76.9096	124.2756	47.366
76.847	124.2888	47.4418
76.8282	124.2609	47.4327
76.8144	124.3024	47.4881
76.9122	124.247	47.3348
76.7607	124.305	47.5444
76.6126	124.2739	47.6613
76.7676	124.2332	47.4656
76.7278	124.2444	47.5166
76.7977	124.2562	47.4585
76.8611	124.2453	47.3842
76.8689	124.1907	47.3218
76.8178	124.1933	47.3755
76.8083	124.2132	47.4049
76.7321	124.1994	47.4673
76.7641	124.1907	47.4266
76.7918	124.176	47.3842
76.8197	124.1734	47.3537
76.8187	124.1907	47.372
76.7866	124.1275	47.3409
76.8923	124.079	47.1867
76.9044	124.0851	47.1807
76.8758	124.124	47.2482
76.9183	124.1492	47.2309
76.9702	124.0981	47.1278
76.9395	124.0668	47.1273
76.9174	124.1145	47.1971
76.804	124.1258	47.3218
76.9391	124.111	47.172
77.0326	124.1535	47.1209
77.0638	124.092	47.0282
77.0716	124.1301	47.0585
77.0283	124.1717	47.1434
76.9339	124.2202	47.2863
76.7906	124.2536	47.4629
76.8577	124.2565	47.3989
76.8663	124.1803	47.314
76.8265	124.1093	47.2828
76.8438	124.0825	47.2387
76.8551	124.0937	47.2387
76.8464	124.1007	47.2543
76.8758	124.1336	47.2577
76.9272	124.1646	47.2374
76.9746	124.1076	47.133
77.0101	124.1734	47.1633
76.9494	124.1604	47.211
76.9936	124.1777	47.1841
76.8074	124.2392	47.4318
76.5927	124.2357	47.6431
76.6931	124.2357	47.5426
76.7519	124.295	47.5431
76.7078	124.2479	47.54
76.7685	124.234	47.4656
76.7858	124.2262	47.4404
76.7979	124.2297	47.4318
76.8395	124.2479	47.4084
76.8299	124.2002	47.3703
76.7728	124.215	47.4422
76.7677	124.2826	47.5149
76.7399	124.2695	47.5296
76.7382	124.3223	47.5842
76.7598	124.3509	47.5911
76.7459	124.3994	47.6535
76.7589	124.3717	47.6128
76.7936	124.3648	47.5712
76.7754	124.4479	47.6725
76.7951	124.4306	47.6356
76.7892	124.4981	47.7089
76.7373	124.48	47.7427
76.7295	124.5631	47.8336
76.6602	124.5241	47.8639
76.5693	124.6003	48.031
76.4593	124.6332	48.1739
76.6117	124.6367	48.025
76.5651	124.6473	48.0822
76.4706	124.7242	48.2536
76.8569	124.4408	47.5839

Table A.4: 72 Hour Test			82.5764	122.8344	40.258	83.31	122.5714	39.2614
minimum	maximum	height	82.5886	122.8467	40.2581	83.2632	122.554	39.2908
mmHg	mmHg	mmHg	82.625	122.89	40.265	83.2767	122.5552	39.2785
82.1219	121.3403	39.2184	82.6588	122.9151	40.2564	83.2451	122.5298	39.2847
82.0284	120.9009	38.8725	82.7004	122.9775	40.2771	83.2165	122.5081	39.2917
81.9392	120.8974	38.9583	82.7082	123.0061	40.2979	83.1619	122.4674	39.3055
81.8329	120.9238	39.0908	82.6856	122.9775	40.2919	83.1325	122.4458	39.3133
81.7937	120.9875	39.1938	83.1351	122.8242	39.6891	83.1108	122.4233	39.3124
81.7192	121.0195	39.3003	83.1833	122.8803	39.6969	83.1152	122.4423	39.3272
81.6776	121.0732	39.3956	83.2173	122.9351	39.7177	83.1108	122.4302	39.3194
81.6742	121.0828	39.4086	83.2451	122.9593	39.7143	83.1375	122.4618	39.3243
81.3103	121.7687	40.4585	83.3135	122.9879	39.6744	83.1048	122.4735	39.3687
81.5529	120.9044	39.3514	83.2251	122.9567	39.7316	83.0719	122.4648	39.393
81.5651	120.9477	39.3826	83.181	122.9498	39.7688	83.0719	122.4622	39.3904
81.5008	120.9564	39.4555	83.1792	122.9203	39.7411	83.0874	122.4692	39.3817
81.4508	120.9381	39.4874	83.1758	122.9342	39.7584	83.0952	122.4848	39.3895
81.4369	120.9468	39.5099	83.1675	122.9454	39.778	83.0918	122.4969	39.4051
81.5036	121.0724	39.5688	83.1723	122.9437	39.7714	83.0814	122.5081	39.4268
81.6447	121.2707	39.6259	83.1273	122.9351	39.8078	83.0785	122.49	39.4115
81.7694	121.4032	39.6337	83.103	122.9177	39.8147	83.084	122.5263	39.4423
81.8656	121.5478	39.6822	83.1342	122.9567	39.8225	83.0424	122.5367	39.4943
81.9608	121.6699	39.7091	83.1351	122.9576	39.8225	83.0727	122.5506	39.4778
81.98	121.7316	39.7515	83.0883	122.9273	39.839	83.0684	122.5618	39.4934
82.0491	121.8413	39.7922	83.0294	122.8935	39.8641	83.0545	122.5653	39.5108
82.0777	121.8872	39.8095	82.9605	122.8899	39.9295	83.0364	122.574	39.5376
82.0448	121.9418	39.897	82.9662	122.8866	39.9204	83.0684	122.5731	39.5047
82.0691	121.9678	39.8987	82.9636	122.8684	39.9048	83.0653	122.6116	39.5463
82.0318	121.979	39.9472	82.8943	122.8372	39.9429	83.1195	122.6597	39.5402
82.063	122.0206	39.9576	82.8831	122.8	39.9169	83.1481	122.6839	39.5359
82.095	122.0916	39.9966	82.8614	122.7783	39.9169	83.1385	122.6822	39.5437
82.1016	122.1156	40.014	82.864	122.7991	39.9351	83.1472	122.6857	39.5385
82.0994	122.154	40.0546	82.845	122.7801	39.9351	83.1143	122.6675	39.5532
82.1461	122.2094	40.0632	82.7922	122.7525	39.9603	83.1273	122.7125	39.5852
82.1886	122.2509	40.0624	82.8095	122.7671	39.9576	83.1186	122.7082	39.5896
82.1669	122.2847	40.1178	82.7965	122.7489	39.9524	83.1314	122.7349	39.6035
82.1323	122.2475	40.1152	82.832	122.8606	40.0286	83.1299	122.7394	39.6095
82.1479	122.283	40.1351	82.8268	122.8736	40.0468	83.1481	122.761	39.6129
82.1981	122.3297	40.1317	82.8684	122.8918	40.0234	83.1472	122.787	39.6398
82.2379	122.4129	40.175	82.8415	122.8935	40.052	83.1524	122.7861	39.6337
82.2813	122.4574	40.1761	82.8164	122.8589	40.0425	83.181	122.8242	39.6432
82.3046	122.4787	40.1741	82.7679	122.819	40.0511	83.2381	122.8121	39.574
82.3652	122.5636	40.1983	82.801	122.8424	40.0413	84.6973	122.4631	37.7658
82.3921	122.5991	40.207	82.7887	122.8285	40.0399	84.4113	122.6028	38.1914
82.3851	122.6753	40.2901	82.7532	122.8043	40.0511	84.5215	122.5783	38.0568
82.5064	122.735	40.2286	82.7298	122.7749	40.0451	84.5743	122.6198	38.0455
82.528	122.7714	40.2434	83.4079	122.6129	39.2051	84.1552	122.6822	38.527
82.5618	122.8026	40.2408	83.3646	122.593	39.2284	84.1119	122.6779	38.566

84.1102	122.6657	38.5556	86.8613	123.4512	36.5898	85.5295	124.2929	38.7634
84.1136	122.6857	38.572	86.838	123.4642	36.6262	84.9926	124.2756	39.283
84.1162	122.7108	38.5946	85.83	123.4971	37.6671	84.602	124.215	39.6129
84.1448	122.7471	38.6024	85.6637	123.5066	37.8429	84.4981	124.3873	39.8892
84.1497	122.7684	38.6187	85.7243	123.5516	37.8273	84.2054	124.2583	40.0529
84.1595	122.7757	38.6162	85.7347	123.5854	37.8507	84.3925	124.4505	40.058
84.2037	122.8459	38.6422	85.7327	123.6273	37.8946	84.3561	124.4704	40.1143
84.2808	122.8744	38.5937	85.707	123.6478	37.9408	84.3864	124.4635	40.0771
84.2998	122.9134	38.6136	85.6464	123.6642	38.0178	84.4466	124.4923	40.0457
84.2929	122.9654	38.6725	85.5996	123.6893	38.0897	84.4436	124.4851	40.0416
84.3137	122.981	38.6673	85.5468	123.6581	38.1113	84.4072	124.5042	40.097
85.707	122.7965	37.0895	85.5598	123.6885	38.1287	84.4011	124.5103	40.1091
85.9538	122.7957	36.8419	85.5962	123.7179	38.1217	84.3734	124.4981	40.1247
86.0127	122.7965	36.7838	85.5814	123.7447	38.1633	84.3336	124.5397	40.2061
86.0473	122.8407	36.7933	85.6067	123.7752	38.1685	84.3422	124.5544	40.2122
86.0084	122.8511	36.8427	85.5936	123.8088	38.2153	84.3414	124.5648	40.2234
86.0421	122.8848	36.8427	85.6291	123.8088	38.1798	84.2915	124.5725	40.2809
86.1192	122.9342	36.815	85.6438	123.8158	38.172	84.3111	124.5579	40.2468
86.1331	122.9749	36.8418	85.6602	123.8218	38.1616	84.3241	124.5917	40.2676
86.1556	122.9792	36.8236	85.6481	123.8383	38.1901	84.3613	124.6194	40.2581
86.115	123.0009	36.886	85.6429	123.8694	38.2265	84.441	124.6505	40.2096
86.1694	123.0026	36.8332	85.6464	123.8894	38.243	84.389	124.6705	40.2815
86.1894	122.9991	36.8098	85.656	123.8924	38.2364	84.4427	124.7042	40.2616
86.1062	123.0104	36.9042	85.694	123.9292	38.2352	84.4029	124.7276	40.3248
86.1227	123.0078	36.8851	85.7087	123.9465	38.2378	84.6571	124.6614	40.0043
86.0854	123.0208	36.9354	85.7018	123.9881	38.2863	85.5598	124.5293	38.9695
86.0984	123.0234	36.925	85.7157	124.0002	38.2845	85.8551	124.538	38.6829
86.127	123.0502	36.9232	85.7425	123.9907	38.2482	85.8352	124.5293	38.6941
86.0789	123.0353	36.9564	85.7295	124.0011	38.2715	85.8906	124.5406	38.65
86.088	123.0381	36.9501	85.688	124.0019	38.314	85.8906	124.5501	38.6595
86.1911	123.0459	36.8548	85.6437	124.0087	38.365	85.9555	124.5995	38.6439
86.2595	123.071	36.8115	85.6438	124.0288	38.385	86.0032	124.628	38.6249
86.3643	123.1074	36.7431	85.7313	124.053	38.3218	85.9749	124.6139	38.6389
86.3799	123.1386	36.7587	85.7287	124.1041	38.3755	85.9893	124.6497	38.6604
86.4258	123.1446	36.7189	85.7538	124.1613	38.4075	85.9382	124.6826	38.7444
86.5279	123.1784	36.6505	85.7685	124.1708	38.4023	85.9209	124.6878	38.7669
86.544	123.2071	36.6631	85.8274	124.1786	38.3512	86.0231	124.7276	38.7045
86.5461	123.2148	36.6686	85.8386	124.1976	38.359	86.0032	124.7683	38.7652
86.5167	123.2234	36.7067	85.8551	124.1981	38.343	86.0621	124.738	38.676
86.6085	123.2078	36.5994	85.8993	124.215	38.3157	85.8456	124.7848	38.9392
86.6959	123.2737	36.5777	86.3548	124.0885	37.7338	85.9168	124.8244	38.9076
86.6899	123.2892	36.5994	86.398	124.0946	37.6965	86.0638	124.8393	38.7755
86.7141	123.3239	36.6098	86.4483	124.131	37.6827	86.0603	124.8636	38.8033
86.6985	123.3265	36.6279	86.4665	124.1976	37.7312	86.1175	124.874	38.7565
86.773	123.3894	36.6164	86.4387	124.2626	37.8238	86.3409	124.9103	38.5694
86.8388	123.4165	36.5777	86.4976	124.273	37.7754	86.3799	124.9259	38.5461
86.8709	123.4235	36.5526	85.8481	124.1558	38.3077	86.3115	124.9164	38.605

86.2898	124.9112	38.6214	87.0674	125.1364	38.0689	88.453	125.3745	36.9215
86.3801	124.9442	38.5641	87.0692	125.1554	38.0862	88.5578	125.3745	36.8167
86.34	124.9545	38.6145	86.9679	125.1467	38.1789	88.563	125.3892	36.8262
86.3392	124.9381	38.5989	87.0047	125.1248	38.1201	88.6253	125.4126	36.7873
86.3192	124.9458	38.6266	86.928	125.1719	38.2438	88.7102	125.436	36.7258
86.3773	124.9528	38.5755	86.9497	125.1762	38.2265	88.6062	125.4305	36.8243
86.4058	124.9701	38.5642	86.9211	125.1658	38.2447	88.6643	125.4412	36.7769
86.4405	124.9675	38.527	86.9445	125.1944	38.2499	88.7673	125.4438	36.6764
86.5046	124.9658	38.4612	86.9194	125.2359	38.3166	88.815	125.4689	36.6539
86.5002	124.9857	38.4854	86.9133	125.2455	38.3322	88.7977	125.4585	36.6608
86.5343	125.0085	38.4742	86.9142	125.2507	38.3365	88.7769	125.4715	36.6946
86.5089	124.9796	38.4707	86.8977	125.2385	38.3408	88.7639	125.4905	36.7267
86.5539	124.9917	38.4378	86.9488	125.2368	38.288	88.757	125.4897	36.7327
86.5262	125.0013	38.4751	87.0259	125.2645	38.2386	88.8405	125.5282	36.6878
86.5219	125.0169	38.495	86.7124	125.3364	38.624	88.9466	125.5581	36.6115
86.5557	125.0454	38.4898	86.6388	125.358	38.7193	88.9501	125.5667	36.6167
86.5288	125.0446	38.5158	86.6145	125.3442	38.7297	88.9908	125.5685	36.5777
86.4846	125.0298	38.5452	86.5738	125.3797	38.8059	89.1094	125.5763	36.4669
86.2031	125.0807	38.8777	86.4691	125.3927	38.9236	89.1155	125.6378	36.5223
86.1054	125.0922	38.9868	86.3677	125.4126	39.0449	88.9726	125.6204	36.6479
86.0248	125.1225	39.0977	86.2885	125.3891	39.1005	88.9215	125.6473	36.7258
85.9451	125.1667	39.2215	86.2933	125.3563	39.063	88.8427	125.6637	36.821
85.8811	125.2065	39.3254	86.2465	125.3632	39.1167	88.7709	125.7009	36.93
85.875	125.2281	39.3531	86.1599	125.3979	39.238	88.6825	125.7044	37.022
86.5418	125.0939	38.5521	86.1235	125.3771	39.2536	88.5794	125.7287	37.1493
87.1073	125.0524	37.9451	86.0387	125.4308	39.3921	88.5188	125.746	37.2272
87.0417	125.042	38.0003	86.0664	125.4256	39.3592	88.5084	125.7711	37.2627
87.1679	125.0463	37.8784	86.1192	125.436	39.3168	88.4946	125.8127	37.3181
87.1575	125.0359	37.8784	86.0868	125.4534	39.3666	88.5093	125.8413	37.332
87.1012	125.0246	37.9234	86.0924	125.4403	39.348	88.5751	125.8664	37.2913
87.0172	125.0679	38.0507	86.1028	125.462	39.3592	88.4494	125.9105	37.4612
86.0153	125.223	39.2077	86.0785	125.4472	39.3687	88.3257	125.933	37.6074
86.0421	125.216	39.1739	86.0742	125.4386	39.3644	88.3179	125.9391	37.6212
86.0621	125.2524	39.1903	86.1668	125.4273	39.2605	88.4036	125.9478	37.5441
86.056	125.2375	39.1816	86.2326	125.4239	39.1912	88.2919	125.9876	37.6957
86.0387	125.2307	39.1921	86.2326	125.4265	39.1938	88.4539	125.9019	37.448
86.0369	125.2411	39.2042	86.2286	125.4578	39.2291	88.7422	125.843	37.1008
86.0032	125.2663	39.2631	86.2422	125.4481	39.2059	88.705	125.8612	37.1562
85.9192	125.3087	39.3895	86.2794	125.4897	39.2103	88.7357	125.8771	37.1414
86.101	125.2541	39.1531	86.2223	125.4983	39.2761	88.6279	125.8681	37.2402
86.8397	125.1069	38.2672	86.4734	125.4776	39.0042	88.4019	125.862	37.4601
86.9514	125.113	38.1616	88.188	125.3139	37.1259	88.4746	125.8664	37.3917
86.8655	125.1186	38.2531	88.3794	125.2983	36.9189	88.5119	125.882	37.3701
86.9679	125.1138	38.146	88.453	125.3243	36.8713	88.472	125.901	37.429
86.9705	125.0905	38.12	88.3983	125.3248	36.9265	88.3664	125.9512	37.5848
87.006	125.1009	38.0949	88.537	125.3563	36.8193	88.3725	125.9616	37.5892
87.0718	125.1286	38.0568	88.5292	125.3503	36.8211	88.3745	125.966	37.5916

88.2685	125.9755	37.7069	86.3158	126.3349	40.0191	88.2928	126.1972	37.9044
88.2391	126.037	37.7979	86.3546	126.2911	39.9365	88.2036	126.1859	37.9823
88.1404	126.0404	37.9	86.2733	126.3236	40.0503	88.1187	126.2076	38.0888
88.1733	126.0344	37.8611	86.2153	126.3401	40.1247	88.2547	126.2101	37.9554
88.0659	126.0326	37.9667	86.7618	126.263	39.5012	88.3127	126.2171	37.9044
88.0243	126.0759	38.0516	88.2062	125.9686	37.7624	88.5517	126.205	37.6532
87.9118	126.0508	38.1391	88.214	125.9928	37.7788	88.2989	126.2162	37.9174
88.0107	126.0453	38.0346	88.3447	125.9703	37.6255	88.2382	126.192	37.9537
87.9325	126.0716	38.1391	88.2322	126.0145	37.7823	88.2027	126.2015	37.9988
87.8581	126.0647	38.2066	88.3005	125.9757	37.6752	88.1421	126.2032	38.0611
87.7481	126.0638	38.3157	88.1066	126.0214	37.9148	88.2642	126.2015	37.9373
87.6251	126.0967	38.4716	88.0936	126.0283	37.9347	88.201	126.181	37.98
87.5983	126.0907	38.4924	88.3318	126.0439	37.7121	88.4201	126.1998	37.7797
87.4857	126.0863	38.6006	88.046	126.0881	38.0421	88.5509	126.1954	37.6446
87.4459	126.0586	38.6127	87.9568	126.1002	38.1434	88.2417	126.2327	37.991
87.3818	126.0794	38.6976	87.9274	126.0811	38.1538	88.1239	126.2396	38.1157
87.5843	126.0577	38.4733	87.8771	126.0933	38.2161	88.2642	126.2431	37.9789
87.5524	126.0552	38.5028	88.1252	126.1052	37.98	88.0503	126.2673	38.217
87.2216	126.0681	38.8466	88.1664	126.1392	37.9728	88.104	126.2803	38.1763
87.193	126.0907	38.8976	87.962	126.1166	38.1546	88.378	126.255	37.877
87.1887	126.0837	38.895	88.0815	126.1236	38.0421	88.1716	126.2587	38.0871
87.2389	126.1114	38.8725	88.0555	126.1244	38.0689	88.4365	126.2396	37.8031
87.2069	126.1028	38.8959	88.1222	126.0993	37.9771	88.6383	126.2535	37.6151
87.3324	126.1114	38.779	88.2313	126.114	37.8827	88.802	126.2171	37.4151
87.3606	126.1123	38.7517	88.1309	126.1123	37.9814	88.737	126.2283	37.4913
87.2761	126.1149	38.8388	88.149	126.1227	37.9737	88.6002	126.2405	37.6403
87.2787	126.0915	38.8128	87.6618	126.1405	38.4786	88.4625	126.2439	37.7814
87.1843	126.1201	38.9357	87.6892	126.1435	38.4543	88.3629	126.2543	37.8914
87.1289	126.153	39.0241	87.8702	126.1392	38.269	88.5005	126.2523	37.7519
87.1289	126.1998	39.0708	87.7879	126.127	38.3391	88.3456	126.2621	37.9165
87.1731	126.2015	39.0284	87.6433	126.1366	38.4932	88.7266	126.2604	37.5337
87.0865	126.179	39.0925	87.7923	126.1097	38.3174	88.6149	126.2509	37.6359
87.1219	126.2004	39.0785	87.7187	126.14	38.4214	88.8375	126.2638	37.4264
87.0501	126.2128	39.1626	87.5766	126.1712	38.5946	89.0531	126.3115	37.2584
86.8536	126.2638	39.4103	87.6671	126.1757	38.5086	89.3233	126.302	36.9787
86.9228	126.2587	39.3358	87.3376	126.2864	38.9487	88.9994	126.3331	37.3337
86.9124	126.2517	39.3393	86.8518	126.4249	39.5731	89.2563	126.3193	37.063
86.8717	126.2751	39.4034	86.9029	126.3972	39.4943	89.1977	126.3392	37.1415
86.7799	126.2907	39.5108	87.9906	126.2431	38.2525	89.1666	126.3305	37.164
86.7817	126.2994	39.5177	88.5041	126.1729	37.6688	89.1648	126.3626	37.1977
86.7836	126.2937	39.5102	88.4469	126.1547	37.7078	88.9509	126.3868	37.4359
86.754	126.3184	39.5644	88.5058	126.1582	37.6524	89.0687	126.3782	37.3095
86.7055	126.2968	39.5913	88.3481	126.1801	37.832	88.7266	126.3513	37.6247
86.663	126.2942	39.6311	88.3508	126.1928	37.842	88.4305	126.25	37.8195
86.5323	126.3106	39.7783	88.3777	126.1902	37.8126	88.0926	126.2788	38.1862
86.5089	126.308	39.7991	88.3491	126.1824	37.8334	88.0486	126.2881	38.2395
86.3833	126.3219	39.9385	88.3664	126.1911	37.8247	87.8953	126.2768	38.3815

88.4591	126.2249	37.7658	88.2859	126.3167	38.0308	87.7568	126.4041	38.6474
88.3993	126.2439	37.8446	88.1335	126.3288	38.1953	87.727	126.4127	38.6856
88.3508	126.2656	37.9148	87.51	126.3756	38.8656	87.7992	126.3972	38.598
88.3638	126.2708	37.907	87.5351	126.3418	38.8067	87.8537	126.4396	38.5859
88.4539	126.269	37.8152	87.7117	126.3002	38.5885	87.9828	126.4492	38.4664
88.5701	126.2567	37.6867	87.5792	126.308	38.7288	87.9403	126.4691	38.5287
88.3231	126.2587	37.9356	88.7474	126.3106	37.5632	88.0157	126.4656	38.4499
88.2651	126.2734	38.0083	87.7182	126.3651	38.6469	88.0295	126.457	38.4274
88.2201	126.2959	38.0758	87.9481	126.3695	38.4214	88.0139	126.47	38.456
88.2625	126.3054	38.0429	88.0772	126.3652	38.288	87.9869	126.4558	38.4689
88.02	126.3167	38.2967	88.162	126.347	38.185	87.91	126.4812	38.5712
88.1187	126.3375	38.2187	88.2989	126.353	38.0542	87.9187	126.4916	38.5729
88.0252	126.3331	38.3079	88.2911	126.4223	38.1313	87.93	126.5063	38.5764
87.9226	126.3563	38.4337	88.5231	126.4405	37.9174	87.8485	126.4855	38.637
88.0971	126.3409	38.2438	88.4928	126.431	37.9382	87.6719	126.4994	38.8275
88.1473	126.3383	38.191	88.504	126.4479	37.9439	87.8815	126.4795	38.598
88.2798	126.3375	38.0577	88.653	126.3877	37.7347	87.9724	126.4734	38.501
88.3231	126.3331	38.01	88.4738	126.3963	37.9226	87.9129	126.4743	38.5614
88.3725	126.3279	37.9555	88.5993	126.3712	37.7719	87.8373	126.4803	38.6431
88.3603	126.3435	37.9832	88.4305	126.4154	37.9849	87.8607	126.5003	38.6396
88.3872	126.3626	37.9754	88.9414	126.3989	37.4575	87.9256	126.521	38.5954
88.2908	126.3536	38.0628	88.9319	126.4258	37.4939	87.7628	126.5375	38.7747
88.1984	126.3678	38.1694	88.9613	126.4665	37.5052	87.781	126.5228	38.7418
88.2711	126.347	38.0758	88.9515	126.469	37.5176	87.8304	126.5158	38.6855
88.4296	126.3375	37.9078	87.8312	126.4318	38.6006	87.8667	126.5158	38.6491
88.4019	126.366	37.9641	87.5368	126.4448	38.908	87.6698	126.5034	38.8336
88.2365	126.3608	38.1243	87.7628	126.3998	38.637	87.7819	126.5158	38.734
88.1949	126.3652	38.1702	87.5533	126.4622	38.9089	87.7204	126.528	38.8076
88.621	126.3427	37.7217	87.4866	126.4769	38.9903	87.7949	126.5236	38.7288
88.3648	126.3536	37.9888	87.4658	126.4336	38.9678	87.7853	126.5271	38.7418
88.479	126.3236	37.8446	87.5481	126.4362	38.8881	87.6788	126.528	38.8492
88.408	126.3349	37.9269	87.6654	126.4541	38.7887	88.1283	126.5366	38.4084
88.3491	126.3444	37.9953	87.5472	126.4881	38.9409	87.7238	126.5418	38.818
88.2737	126.3496	38.0758	87.6693	126.4474	38.7781	87.7085	126.5466	38.838
88.188	126.3253	38.1373	87.4381	126.4691	39.031	87.8702	126.5098	38.6396
88.1378	126.3496	38.2118	87.7481	126.4154	38.6673	87.8737	126.5081	38.6344
88.4669	126.3427	37.8758	87.9559	126.4154	38.4595	87.9213	126.5158	38.5946
88.3292	126.3227	37.9936	87.923	126.4033	38.4803	88.0044	126.5184	38.514
88.0618	126.3501	38.2883	87.7464	126.4379	38.6915	87.8529	126.5522	38.6993
87.9776	126.3297	38.3521	87.6996	126.4457	38.7461	87.9291	126.528	38.5989
87.917	126.3158	38.3988	87.7667	126.4188	38.6522	87.9516	126.5349	38.5833
88.2426	126.2994	38.0568	88.0139	126.4206	38.4066	87.5351	126.5825	39.0475
88.6063	126.289	37.6827	88.0278	126.405	38.3772	87.6856	126.5527	38.8671
88.6556	126.2846	37.629	87.9724	126.4379	38.4655	87.5498	126.5635	39.0137
88.4608	126.3002	37.8394	87.9577	126.418	38.4603	87.6416	126.5912	38.9496
88.4461	126.3245	37.8784	87.852	126.4275	38.5755	87.9092	126.5869	38.6777
88.2723	126.3193	38.047	87.8425	126.3963	38.5539	88.1941	126.586	38.3919

88.2382	126.5912	38.353	87.7524	126.7739	39.0215	87.4545	126.9488	39.4943
88.3309	126.6042	38.2733	87.6026	126.7704	39.1678	87.5048	126.9497	39.4449
88.3274	126.605	38.2776	87.846	126.7505	38.9046	87.4563	126.9887	39.5324
88.2609	126.6294	38.3685	87.613	126.7635	39.1505	87.5784	126.9826	39.4042
88.3569	126.6284	38.2716	87.6087	126.7652	39.1566	87.4104	126.9835	39.5731
88.2963	126.657	38.3607	87.868	126.7404	38.8724	87.3662	127.0146	39.6484
88.0902	126.6206	38.5305	87.7931	126.7445	38.9513	87.6061	127.0051	39.399
88.3248	126.6354	38.3105	87.5992	126.7782	39.1791	87.5517	127.0037	39.452
88.3465	126.638	38.2915	88.0521	126.7401	38.6881	87.6823	127.0528	39.3705
88.472	126.6622	38.1902	87.8867	126.767	38.8803	87.7342	127.0389	39.3047
88.2642	126.6709	38.4066	87.658	126.7756	39.1176	87.729	127.0302	39.3012
88.5516	126.6637	38.1122	87.6494	126.7895	39.1401	87.7645	127.0398	39.2752
88.4954	126.6665	38.1711	87.4424	126.7921	39.3497	87.4814	127.0493	39.5679
88.6201	126.6804	38.0603	87.4989	126.7906	39.2917	87.3238	127.1082	39.7844
88.3257	126.6674	38.3417	87.4251	126.8103	39.3852	87.4511	127.0649	39.6138
88.7587	126.6882	37.9295	87.4935	126.8111	39.3176	87.2073	127.076	39.8687
88.666	126.715	38.049	87.4234	126.8207	39.3973	88.4625	127.045	38.5824
88.7214	126.6735	37.952	87.3523	126.8674	39.5151	87.9966	127.0354	39.0388
89.1761	126.7254	37.5493	87.5004	126.851	39.3506	88.007	127.0372	39.0301
88.5057	126.7412	38.2355	87.7022	126.8224	39.1202	87.7914	127.0354	39.244
87.729	126.5635	38.8344	87.7264	126.8345	39.1081	87.8849	127.0683	39.1834
87.7005	126.5643	38.8639	87.5931	126.8249	39.2318	87.9568	127.0458	39.089
87.7585	126.5721	38.8136	87.7117	126.7878	39.076	88.0685	127.038	38.9695
87.6788	126.6128	38.934	87.7524	126.7826	39.0301	87.8125	126.9016	39.0891
87.7801	126.6319	38.8518	87.7628	126.7722	39.0094	88.0624	126.8631	38.8007
87.684	126.6224	38.9383	87.7594	126.7618	39.0024	88.1239	126.9029	38.779
87.6	126.6371	39.0371	87.6953	126.7869	39.0916	88.1672	126.896	38.7288
87.6539	126.6294	38.9755	87.7827	126.7687	38.986	88.1265	126.9038	38.7773
87.8078	126.6466	38.8388	87.6875	126.7895	39.102	88.1257	126.8925	38.7669
87.7706	126.6457	38.8751	87.7852	126.7941	39.0089	88.3292	126.9185	38.5894
87.5957	126.6648	39.0691	87.8572	126.7904	38.9332	88.369	126.9246	38.5556
87.6199	126.6787	39.0587	87.9109	126.7956	38.8847	88.4194	126.9412	38.5218
87.497	126.7202	39.2233	87.6468	126.7964	39.1496	88.2538	126.9341	38.6803
87.3801	126.7029	39.3228	87.6234	126.8163	39.1929	88.143	126.9428	38.7998
87.5879	126.7098	39.1219	87.6624	126.8345	39.1722	88.1464	126.9159	38.7695
87.4865	126.7069	39.2203	87.7282	126.8337	39.1055	88.1638	126.9445	38.7807
87.393	126.7194	39.3263	87.7845	126.8354	39.0509	87.7715	126.9402	39.1687
87.5446	126.7202	39.1756	87.7667	126.8602	39.0935	87.8122	126.9246	39.1124
87.5844	126.7185	39.1341	87.6693	126.8804	39.2111	87.8009	126.9332	39.1323
87.5221	126.7358	39.2137	87.7031	126.8735	39.1704	87.8565	126.9236	39.0671
87.5879	126.7453	39.1574	87.5654	126.8718	39.3064	88.0789	126.9255	38.8466
87.742	126.741	38.999	87.5576	126.864	39.3064	88.0356	126.9203	38.8847
87.7109	126.7297	39.0189	87.4519	126.8458	39.3939	88.0079	126.9306	38.9228
87.5412	126.7562	39.2151	87.374	126.8622	39.4882	88.2192	126.928	38.7089
87.4511	126.7704	39.3194	87.4935	126.8874	39.3938	88.2434	126.9289	38.6855
87.6165	126.728	39.1115	87.4989	126.8972	39.3983	88.1508	126.9177	38.7669
87.8018	126.7497	38.9479	87.4355	126.9255	39.49	88.1005	126.9272	38.8266

87.8512	126.8945	39.0433	88.2954	127.0328	38.7374	86.3903	127.0813	40.6911
88.0624	126.8995	38.837	88.2287	127.025	38.7963	86.2976	127.1004	40.8028
87.9022	126.8778	38.9756	88.2371	127.0293	38.7922	86.2811	127.1238	40.8426
87.813	126.8995	39.0864	88.1698	127.0268	38.8569	86.1764	127.18	41.0037
87.9516	126.8891	38.9375	88.0728	127.0172	38.9444	86.0188	127.1922	41.1734
87.6754	126.8882	39.2129	87.9464	127.032	39.0856	85.974	127.2266	41.2526
87.5515	126.883	39.3315	87.839	127.077	39.238	85.9218	127.2502	41.3284
87.3229	126.9029	39.58	87.9109	127.0553	39.1444	85.8447	127.2718	41.4271
87.5446	126.8934	39.3488	87.8702	127.0398	39.1696	85.6931	127.3541	41.661
87.8363	126.891	39.0547	87.9057	127.0415	39.1358	85.6152	127.361	41.7458
87.7135	126.8744	39.1609	87.9118	127.0458	39.1341	85.6802	127.2528	41.5726
87.8234	126.8544	39.031	87.9569	127.0531	39.0961	85.7139	127.2112	41.4973
87.6693	126.864	39.1947	87.7853	127.0796	39.2943	85.7815	127.2303	41.4488
87.6788	126.9194	39.2406	87.9776	127.0034	39.0258	85.819	127.2619	41.4429
87.6407	126.9151	39.2743	88.1135	126.9566	38.8431	85.8135	127.2614	41.4479
87.7576	126.8891	39.1315	88.2088	126.9705	38.7617	85.8049	127.2485	41.4436
87.7368	126.8822	39.1453	88.1802	126.9705	38.7903	85.7893	127.2424	41.4531
88.356	126.8883	38.5324	87.9577	126.9722	39.0146	85.8473	127.2744	41.4271
88.0356	126.8596	38.824	87.8633	126.9662	39.1029	85.649	127.2285	41.5796
87.9871	126.8466	38.8595	88.0257	126.9958	38.9702	85.6369	127.2259	41.5891
87.9196	126.8596	38.9401	87.8191	126.9921	39.173	85.6724	127.2407	41.5683
88.0139	126.851	38.837	87.7316	126.9939	39.2622	85.6833	127.2592	41.5759
88.1421	126.8804	38.7383	87.6442	126.993	39.3488	85.6983	127.2831	41.5848
88.1378	126.8726	38.7348	87.574	126.9921	39.4181	85.7148	127.3004	41.5856
88.1153	126.864	38.7487	87.5515	127.0198	39.4683	85.7468	127.3368	41.5899
88.1833	126.8813	38.698	87.5307	127.012	39.4813	85.7494	127.348	41.5986
88.1811	126.8874	38.7063	87.529	127.0311	39.5021	85.7269	127.3584	41.6315
88.2937	126.9246	38.6309	87.52	127.054	39.5339	85.7209	127.3359	41.6151
88.2469	126.9315	38.6846	87.5515	127.0571	39.5056	85.6265	127.3411	41.7146
88.2278	126.9549	38.727	87.4078	127.038	39.6303	85.6129	127.3905	41.7776
88.3292	126.9618	38.6327	87.3039	127.0354	39.7316	85.6594	127.4026	41.7432
88.3465	126.9826	38.6361	87.2597	127.0216	39.7619	84.8332	127.3853	42.552
88.4443	126.9783	38.5339	87.1428	127.0198	39.8771	84.2375	127.7576	43.5202
88.5375	126.9914	38.454	87.0839	127.012	39.9282	82.9498	127.5524	44.6027
88.6175	127.0034	38.3859	87.0146	127.0224	40.0078	83.1229	128.2712	45.1482
88.5612	126.9211	38.3599	86.9915	127.0337	40.0422	83.2416	128.3171	45.0755
88.6262	126.9237	38.2975	87.0051	127.0484	40.0433	83.1654	128.3136	45.1482
88.7258	126.9358	38.2101	86.8709	127.0354	40.1646	83.1939	128.3277	45.1338
88.7613	126.9566	38.1953	86.7462	127.0095	40.2633	83.194	128.3552	45.1612
88.7301	126.9783	38.2482	86.6726	127.0233	40.3507	83.213	128.3734	45.1603
88.7353	126.9757	38.2404	86.6985	127.0224	40.3239	83.194	128.3976	45.2036
88.7815	127.0046	38.2232	86.6414	127.0337	40.3923	83.194	128.4054	45.2114
88.7587	127.006	38.2473	86.5695	127.0398	40.4702	83.1688	128.3586	45.1898
88.6695	127.0164	38.3469	86.4964	127.0249	40.5285	83.2329	128.3708	45.1378
88.5465	127.006	38.4595	86.4032	127.0623	40.659	83.2234	128.3448	45.1214
88.4729	127.0294	38.5565	86.3227	127.0605	40.7378	83.2918	128.2781	44.9863
88.4426	127.0432	38.6006	86.5063	127.0753	40.569	83.2814	128.2634	44.982

83.1818	128.2357	45.0538	82.3574	123.5949	41.2375	82.024	125.5927	43.5687
83.1983	128.2539	45.0556	82.5402	123.5793	41.0392	81.9799	125.6802	43.7003
83.284	128.2677	44.9837	82.5332	123.5837	41.0504	82.0621	125.6733	43.6111
83.2797	128.2738	44.9941	82.5139	123.6361	41.1222	82.0084	125.7451	43.7367
83.278	128.2937	45.0157	82.5488	123.7491	41.2003	82.0933	125.7841	43.6908
83.2849	128.2963	45.0114	82.5194	123.7577	41.2384	82.0664	125.7308	43.6645
83.2676	128.3145	45.0469	82.4865	123.7828	41.2964	82.0076	125.7218	43.7142
83.2987	128.3621	45.0634	82.6683	123.8668	41.1985	81.8794	125.7867	43.9073
83.2511	128.3318	45.0807	82.6042	123.9231	41.3189	81.9322	125.8542	43.922
83.2234	128.3656	45.1422	82.6796	123.9422	41.2626	81.8915	125.9417	44.0502
83.2676	128.3508	45.0833	82.7913	123.9604	41.1691	81.7539	126.0413	44.2874
83.1888	128.3474	45.1586	83.11	123.9967	40.8868	81.7608	126.0976	44.3368
83.2312	128.3976	45.1664	83.0001	123.9664	40.9663	81.7106	126.1469	44.4364
83.2745	128.4123	45.1378	82.8424	124.0357	41.1933	81.6266	126.1773	44.5507
83.2624	128.4193	45.1569	82.929	124.0903	41.1613	81.5711	126.2128	44.6416
83.2397	128.3956	45.1559	82.7766	124.1414	41.3648	81.624	126.2587	44.6347
83.2554	128.3993	45.1439	82.8744	124.1535	41.2791	81.5088	126.3634	44.8547
83.2684	128.4253	45.1569	82.7497	124.1968	41.4471	81.4655	126.4457	44.9802
83.2832	128.4158	45.1326	82.8303	124.2332	41.4029	81.3979	126.4396	45.0417
83.2797	128.4054	45.1257	82.9567	124.3102	41.3535	81.3616	126.5037	45.1422
83.3039	128.4106	45.1067	82.8997	124.3425	41.4429	81.3165	126.5003	45.1837
83.2953	128.4071	45.1118	82.819	124.3249	41.506	81.3165	126.5202	45.2036
83.2589	128.4444	45.1855	82.6172	124.363	41.7458	81.3026	126.5378	45.2351
83.2424	128.4334	45.1911	82.5081	124.3596	41.8515	81.1892	126.5929	45.4037
83.2321	128.4314	45.1993	82.6042	124.3838	41.7796	81.2074	126.6743	45.4669
83.2321	128.4426	45.2106	82.6112	124.3994	41.7883	81.1944	126.7168	45.5223
83.2069	128.4677	45.2608	82.6354	124.396	41.7605	81.2074	126.6622	45.4548
83.2095	128.4677	45.2582	82.4631	124.4462	41.9831	81.1468	126.7064	45.5596
83.3031	128.4132	45.1101	82.5156	124.5143	41.9987	81.1529	126.7289	45.576
83.2806	128.3837	45.1032	82.5765	124.5527	41.9762	81.191	126.7652	45.5743
83.2987	128.3517	45.053	82.5072	124.6644	42.1572	81.196	126.8188	45.6227
83.378	128.4361	45.0581	82.3808	124.7649	42.384	81.1849	126.8328	45.6479
83.3827	128.4045	45.0218	82.4206	124.8922	42.4715	81.1659	126.883	45.7172
83.4053	128.4019	44.9967	82.5679	124.8445	42.2767	81.3373	126.8415	45.5041
81.1618	142.1615	60.9997	82.5012	124.9528	42.4516	81.4109	126.8172	45.4063
83.8097	127.8927	44.0831	82.0838	125.0922	43.0084	81.3815	126.8051	45.4236
82.2107	129.8027	47.592	82.1536	125.0834	42.9298	81.3893	126.8328	45.4435
83.7698	127.9698	44.2	82.1366	125.139	43.0023	81.3226	126.87	45.5474
84.1136	127.9776	43.864	82.1661	125.1805	43.0145	81.3246	126.8452	45.5205
84.7875	127.8159	43.0285	82.2951	125.2152	42.9201	81.2975	126.8761	45.5786
84.3405	127.7672	43.4267	82.2821	125.2922	43.0101	81.3728	126.9064	45.5336
84.0365	127.7732	43.7367	82.1738	125.3849	43.211	81.475	126.8908	45.4158
82.254	123.4379	41.1839	82.1548	125.4732	43.3184	81.4646	126.8778	45.4132
82.289	123.4304	41.1414	82.2085	125.4758	43.2673	81.4542	126.941	45.4868
82.2873	123.478	41.1907	82.0681	125.6049	43.5368	81.4005	126.9177	45.5171
82.3505	123.5231	41.1726	82.0007	125.6256	43.625	81.4031	126.9462	45.5431
82.315	123.5889	41.2739	82.147	125.6282	43.4812	81.3942	126.9535	45.5593

81.4811	126.9419	45.4608	81.669	127.4953	45.8263	81.9504	127.8061	45.8557
81.4949	126.928	45.4331	81.6656	127.5094	45.8438	81.947	127.7914	45.8445
81.4612	126.9471	45.4859	81.7478	127.5022	45.7544	82.0292	127.7585	45.7293
81.4127	126.9939	45.5812	81.8153	127.529	45.7137	82.0514	127.7481	45.6967
81.3988	127.0172	45.6184	81.8699	127.5351	45.6652	82.0206	127.7559	45.7353
81.4586	126.9913	45.5327	81.9236	127.4996	45.576	82.0258	127.7602	45.7345
81.4897	126.9739	45.4842	81.9141	127.4632	45.5492	81.9686	127.8191	45.8505
81.5202	126.9844	45.4642	81.8959	127.5048	45.6089	81.9721	127.8901	45.9181
81.52	126.9688	45.4487	81.8863	127.5074	45.621	81.927	127.9586	46.0315
81.5408	126.9679	45.4271	81.8426	127.4865	45.6439	82.0076	127.9785	45.9709
81.5079	126.9506	45.4426	81.8794	127.5334	45.6539	82.1687	127.9958	45.8271
81.4456	126.9532	45.5076	81.9158	127.5221	45.6063	82.224	127.9974	45.7734
81.4179	126.9532	45.5353	81.9426	127.5715	45.6288	82.2708	127.9447	45.6739
81.4412	126.9653	45.524	81.9011	127.5524	45.6513	82.2596	127.9187	45.6591
81.3616	126.9913	45.6297	81.9253	127.4979	45.5725	82.2778	127.9057	45.628
81.3211	127.046	45.7249	81.8863	127.5238	45.6375	82.2353	127.9343	45.699
81.3702	127.0614	45.6912	81.8855	127.5767	45.6912	82.192	128.0079	45.8159
81.3849	127.0727	45.6877	81.803	127.6063	45.8033	82.2371	128.0045	45.7674
81.3711	127.1246	45.7535	81.7937	127.6174	45.8237	82.2397	128.0079	45.7683
81.3772	127.1567	45.7795	81.6906	127.6122	45.9215	82.3086	127.9921	45.6835
81.3564	127.1809	45.8245	81.6733	127.6477	45.9744	82.3947	127.9802	45.5855
81.333	127.1766	45.8436	81.927	127.5498	45.6228	82.3739	127.9629	45.589
81.4386	127.2086	45.77	82.044	127.5126	45.4686	82.4544	127.9646	45.5102
81.4672	127.1913	45.7241	82.0353	127.4866	45.4513	82.5124	127.8927	45.3803
81.4762	127.1905	45.7143	82.0587	127.4979	45.4392	82.3765	127.9248	45.5483
81.4837	127.2078	45.7241	81.9461	127.5654	45.6193	82.3063	128.0183	45.712
81.4386	127.187	45.7483	81.9439	127.616	45.6721	82.2197	128.0512	45.8315
81.4014	127.2537	45.8523	81.953	127.633	45.6799	82.186	128.1075	45.9215
81.4265	127.258	45.8315	81.9478	127.6243	45.6765	82.2381	128.1982	45.9601
81.5235	127.2156	45.692	81.9747	127.594	45.6193	82.1868	128.2019	46.0151
81.5313	127.2104	45.6791	81.9089	127.6113	45.7024	82.1946	128.2487	46.054
81.5573	127.2207	45.6635	81.9322	127.6407	45.7085	82.2275	128.2824	46.0549
81.5581	127.2266	45.6685	81.9193	127.6537	45.7345	82.2466	128.3197	46.0731
81.5443	127.2597	45.7154	81.9037	127.6754	45.7717	82.3531	128.2348	45.8817
81.6179	127.2303	45.6124	81.9113	127.7129	45.8015	82.5246	128.1664	45.6418
81.6569	127.1948	45.5379	81.869	127.7551	45.886	82.5956	128.1517	45.5561
81.6898	127.1948	45.505	81.8145	127.7888	45.9744	82.6116	128.1639	45.5523
81.6222	127.2389	45.6167	81.7824	127.8157	46.0332	82.6034	128.2132	45.6098
81.5824	127.2814	45.699	81.8353	127.8035	45.9683	82.6068	128.2807	45.6739
81.5374	127.3091	45.7717	81.8474	127.8061	45.9588	82.6328	128.2313	45.5985
81.5026	127.3235	45.8209	81.8526	127.7923	45.9397	82.1167	129.235	47.1183
81.4707	127.374	45.9033	81.8552	127.7602	45.9051	82.7662	128.0564	45.2902
81.4741	127.4113	45.9371	81.7906	127.8107	46.02	81.6369	122.3064	40.6694
81.4915	127.4286	45.9371	81.7227	127.8113	46.0887	81.9218	122.3306	40.4088
81.5486	127.4139	45.8652	81.7755	127.8425	46.067	81.8578	122.4224	40.5646
81.6638	127.4502	45.7864	81.8586	127.7984	45.9397	81.8482	122.4111	40.5629
81.7123	127.4268	45.7146	81.9097	127.7732	45.8635	81.7448	122.4997	40.7549

81.6162	122.6796	41.0634	82.1089	122.7887	40.6798	81.7963	121.6266	39.8303
81.5824	122.5757	40.9933	82.0752	122.9366	40.8615	81.7616	121.8335	40.0719
81.598	122.5921	40.9942	82.0699	122.8848	40.8149	81.7218	121.8465	40.1247
81.5928	122.6614	41.0686	81.9496	122.8788	40.9292	81.8047	121.7254	39.9207
81.5478	122.8372	41.2895	81.9729	122.8727	40.8998	81.8301	121.7513	39.9212
81.63	122.8641	41.234	81.9998	122.8519	40.8521	81.8673	121.5911	39.7238
81.6647	122.8363	41.1717	82.0136	122.8026	40.7889	81.8959	121.7348	39.839
81.7008	122.83	41.1293	82.095	122.7861	40.6911	81.8742	121.6543	39.7801
81.7227	122.7359	41.0132	82.044	122.8173	40.7733	81.8283	121.8119	39.9836
81.7677	122.554	40.7863	81.9518	122.8371	40.8852	81.8058	121.7279	39.9221
81.7452	122.5402	40.795	81.4975	121.4343	39.9368	81.8647	121.8067	39.942
81.7495	122.4839	40.7344	81.5997	121.5703	39.9706	81.9457	121.6452	39.6996
81.7287	122.5047	40.7759	81.6395	121.5279	39.8883	81.9911	121.6655	39.6744
81.759	122.5653	40.8062	81.669	121.5634	39.8944	82.0223	121.6283	39.606
81.7192	122.5869	40.8677	81.7062	121.43	39.7238	82.0292	121.8379	39.8086
81.7202	122.7825	41.0623	81.7573	121.4863	39.729	81.9556	121.7461	39.7905
81.7738	122.6943	40.9206	81.7547	121.6197	39.8649	81.8742	121.766	39.8918
81.8491	122.7064	40.8573	81.7369	121.5607	39.8238	81.9158	121.8327	39.9169
81.8967	122.69	40.7933	81.6274	121.6006	39.9732	81.8846	121.7314	39.8468
81.9331	122.6761	40.743	81.5876	121.5902	40.0026	81.8417	121.9113	40.0695
81.94	122.7186	40.7785	81.5503	121.7374	40.1871	81.8656	121.792	39.9264
81.9288	122.7861	40.8573	81.5417	121.8015	40.2598	81.882	121.7617	39.8797
81.9314	122.574	40.6426	81.5382	121.8465	40.3083	81.8335	121.7556	39.9221
81.934	122.8216	40.8876	81.6776	121.8188	40.1412	81.8638	121.8803	40.0165
82.0144	122.7428	40.7284	81.7694	121.6378	39.8684	81.9115	121.7201	39.8086
81.9781	122.7168	40.7387	81.7443	121.7478	40.0035	81.9868	122.0829	40.0962
81.9167	122.8597	40.9431	81.8708	121.7078	39.837	81.8786	121.7859	39.9074
81.8811	122.774	40.8928	81.7997	121.7409	39.9411	82.0604	121.8379	39.7775
81.9218	122.6683	40.7465	81.7997	121.7946	39.9948	82.2038	121.8531	39.6493
81.9374	122.7783	40.8409	81.8205	121.5443	39.7238	82.27	122.6147	40.3447
81.9946	122.7445	40.75	81.9963	121.6352	39.6389	82.2414	121.7582	39.5168
81.9929	122.7428	40.75	82.0916	121.6517	39.5601	82.2284	121.9539	39.7255
82.0241	122.6301	40.606	81.9496	121.7123	39.7628	82.1609	121.6586	39.4978
82.0067	122.6562	40.6495	81.8881	121.6647	39.7766	82.1453	121.8457	39.7004
82.0015	122.7238	40.7222	81.8611	121.7597	39.8986	82.1279	121.7807	39.6528
81.8075	122.147	40.3395	81.759	121.6811	39.9221	82.1409	122.0189	39.8779
82.0232	122.8225	40.7993	81.7911	121.7123	39.9212	82.0479	121.9941	39.9462
81.979	122.7004	40.7214	81.7677	121.7158	39.9481	81.9972	121.9946	39.9974
81.9911	122.8156	40.8244	81.7764	121.6664	39.89	82.0414	121.986	39.9446
82.1228	122.6874	40.5646	81.7755	121.8907	40.1152	82.0474	121.9738	39.9264
82.1509	122.7076	40.5567	81.8266	121.7461	39.9195	82.1461	121.8309	39.6848
82.1591	122.742	40.5828	81.837	121.7348	39.8978	82.2102	121.8032	39.593
82.1513	122.7827	40.6313	81.869	121.7104	39.8414	82.1851	121.9063	39.7212
82.0933	122.8502	40.7569	81.8344	121.6404	39.8061	82.2414	121.8794	39.6381
82.0682	122.8493	40.7811	81.8508	121.7123	39.8615	82.1677	121.7844	39.6167
82.0067	122.7982	40.7915	81.8673	121.6448	39.7775	82.2319	122.0604	39.8286
82.0448	122.7342	40.6893	81.8526	121.6915	39.839	82.2457	121.7807	39.535

82.315	121.9158	39.6008	82.4674	121.7816	39.3142	82.2284	122.0985	39.8701
82.3245	121.8561	39.5315	82.4553	121.6179	39.1626	82.2082	122.0557	39.8475
82.3063	121.8292	39.5229	82.3375	121.7582	39.4207	82.3237	122.1124	39.7887
82.205	121.7937	39.5887	82.2682	121.6768	39.4086	82.3124	122.1046	39.7922
82.1557	121.7591	39.6034	82.3323	121.9695	39.6372	82.3219	122.0795	39.7576
82.1007	121.6364	39.5357	82.2613	121.8509	39.5896	82.2674	122.1288	39.8615
82.1851	121.8223	39.6372	82.263	121.8439	39.5809	82.2596	122.0648	39.8052
82.1357	121.9072	39.7714	82.2469	121.7897	39.5428	82.328	122.063	39.735
82.0976	121.8422	39.7446	82.2059	121.8509	39.645	82.2812	122.1384	39.8571
82.1444	121.9617	39.8173	82.2128	122.0041	39.7913	82.2928	122.098	39.8053
82.1202	121.8768	39.7567	82.1141	121.8699	39.7558	82.2163	122.115	39.8987
82.1687	121.8994	39.7307	82.0249	121.9626	39.9377	82.018	122.0206	40.0026
82.1652	121.7149	39.5497	82.0587	121.8734	39.8147	81.9946	122.1903	40.1957
82.2549	121.7536	39.4987	82.199	121.9124	39.7134	82.0214	122.2648	40.2434
82.3522	121.811	39.4588	82.3055	121.8093	39.5038	81.9851	122.2544	40.2693
82.2985	121.643	39.3445	82.3553	121.8593	39.504	82.0249	122.2483	40.2234
82.3401	121.8309	39.4908	82.2873	121.792	39.5047	82.0821	122.1514	40.0693
82.431	121.876	39.4449	82.244	121.7747	39.5307	82.1791	122.1685	39.9894
82.4354	121.6422	39.2068	82.1964	121.8665	39.6701	82.2128	122.1643	39.9515
82.4146	121.6439	39.2293	82.2985	121.8673	39.5688	82.2552	122.1392	39.884
82.3903	121.6404	39.2501	82.2587	121.7894	39.5307	82.2561	122.1037	39.8476
82.3368	121.7553	39.4185	82.2882	122.0526	39.7645	82.1877	122.147	39.9593
82.3107	121.579	39.2683	82.1773	121.9453	39.7679	82.2622	122.1756	39.9134
82.2717	121.7773	39.5056	82.1034	121.8522	39.7489	82.1704	122.2328	40.0624
82.1972	121.7608	39.5636	82.0968	121.9383	39.8416	82.0847	122.2432	40.1585
82.3323	121.8284	39.496	82.0916	122.0241	39.9325	82.0373	122.246	40.2087
82.2552	121.7184	39.4631	82.1141	122.0163	39.9022	82.0076	122.2483	40.2408
82.2509	121.7989	39.548	82.0994	121.9712	39.8719	81.9998	122.3029	40.3031
82.2951	121.8994	39.6043	82.1721	121.8768	39.7047	81.9513	122.3306	40.3793
82.2587	121.8621	39.6034	82.2613	121.7738	39.5125	81.9296	122.4752	40.5456
82.1932	121.6919	39.4987	82.2076	121.8803	39.6727	82.0414	122.4215	40.3802
82.27	121.669	39.399	82.2812	121.9782	39.6969	82.0561	122.4189	40.3629
82.3219	121.9964	39.6744	82.3051	121.9527	39.6476	82.0344	122.4371	40.4027
82.2137	121.7547	39.5411	82.2925	122.0128	39.7203	82.1695	122.4172	40.2477
82.2838	121.8907	39.6069	82.2102	121.8924	39.6822	82.2839	122.4689	40.1849
82.2345	121.9548	39.7203	82.2544	121.9357	39.6813	82.2102	122.4207	40.2105
82.121	121.9028	39.7818	82.2345	121.9037	39.6692	82.1635	122.4501	40.2867
82.1487	121.8976	39.7489	82.179	121.9834	39.8043	82.1505	122.4778	40.3274
82.1862	121.9042	39.718	82.0881	122.0345	39.9463	82.05	122.5861	40.5361
82.1427	121.8794	39.7368	82.0249	121.9608	39.9359	82.0621	122.6398	40.5776
82.1981	121.798	39.5999	81.9466	121.9976	40.051	82.0474	122.7004	40.653
82.3263	121.9098	39.5835	81.8534	122.0639	40.2105	82.0292	122.522	40.4928
82.4544	121.7712	39.3168	81.9686	122.044	40.0754	82.0065	122.6204	40.6139
82.4648	121.805	39.3402	82.1028	122.0015	39.8987	81.8803	122.5783	40.698
82.5237	121.8933	39.3696	82.1323	121.9842	39.8519	81.9279	122.7047	40.7768
82.4579	121.7452	39.2873	82.2033	122.063	39.8597	81.9738	122.729	40.7552
82.424	121.7835	39.3595	82.1531	122.0128	39.8597	82.0769	122.6657	40.5889

82.1409	122.567	40.4261	82.2223	122.9177	40.6954	82.5384	122.6285	40.0901
82.2544	122.5575	40.3031	82.2319	122.8952	40.6634	82.5055	122.6346	40.1291
82.3366	122.5358	40.1992	82.2449	122.9463	40.7015	82.599	122.6285	40.0295
82.2469	122.5341	40.2871	82.2959	122.9403	40.6443	82.6562	122.593	39.9368
82.2293	122.6458	40.4166	82.3254	122.9247	40.5993	82.6285	122.5644	39.9359
82.1825	122.664	40.4815	82.3421	122.7534	40.4113	82.606	122.5307	39.9247
82.199	122.5289	40.33	82.1877	122.8164	40.6287	82.5964	122.6173	40.0208
82.199	122.5999	40.401	82.2648	122.8667	40.6019	82.5958	122.5217	39.9259
82.1202	122.6623	40.5421	82.2128	122.9342	40.7214	82.6181	122.5696	39.9515
82.0907	122.7246	40.6339	82.1357	122.7697	40.6339	82.5687	122.5012	39.9325
81.9903	122.6571	40.6668	82.1626	122.8095	40.6469	82.5505	122.5376	39.987
82.0998	122.6829	40.5831	82.2908	122.8285	40.5378	82.5947	122.5298	39.9351
82.2197	122.6164	40.3966	82.3011	122.8649	40.5638	82.6164	122.4891	39.8727
82.2752	122.7038	40.4287	82.239	122.7728	40.5338	82.5757	122.541	39.9654
82.2743	122.6649	40.3906	82.2197	122.8589	40.6391	82.4899	122.522	40.0321
82.3418	122.6831	40.3412	82.2353	122.7428	40.5075	82.4134	122.5235	40.1101
82.3947	122.6372	40.2425	82.2526	122.774	40.5213	82.4224	122.5618	40.1394
82.4466	122.7194	40.2728	82.2215	122.7896	40.5681	82.4267	122.5532	40.1265
82.5228	122.6311	40.1083	82.1617	122.729	40.5672	82.3618	122.5748	40.2131
82.565	122.7789	40.214	82.1279	122.6597	40.5317	82.3626	122.5246	40.162
82.5367	122.8554	40.3187	82.121	122.7852	40.6642	82.4068	122.5956	40.1888
82.3055	122.8311	40.5257	82.0373	122.7684	40.7311	82.3817	122.6129	40.2312
82.0058	122.9359	40.9301	82.0275	122.7775	40.75	82.4189	122.6337	40.2148
82.0465	122.8727	40.8262	81.9461	122.7835	40.8374	82.4196	122.5358	40.1162
82.1002	122.9455	40.8452	82.0795	122.7021	40.6227	82.4865	122.6216	40.1351
82.0336	122.9247	40.8911	82.0388	122.7168	40.6781	82.4908	122.5783	40.0875
82.179	122.9714	40.7924	81.9651	122.664	40.6989	82.5904	122.5445	39.9541
82.1967	122.9675	40.7707	82.0249	122.6415	40.6166	82.6848	122.6077	39.923
82.2085	122.9125	40.7041	82.1011	122.7212	40.6201	82.7237	122.5003	39.7766
82.2708	123.0199	40.7491	82.0232	122.7331	40.7099	82.7766	122.4493	39.6727
82.2665	122.9732	40.7067	81.9877	122.8822	40.8946	82.6848	122.4804	39.7957
82.2977	122.9732	40.6755	82.0249	122.8944	40.8695	82.7772	122.468	39.6907
82.2171	123.0156	40.7985	82.1894	122.8251	40.6356	82.7722	122.4605	39.6883
82.1314	123.0095	40.8781	82.4163	122.6493	40.233	82.7047	122.5021	39.7974
82.0933	122.9697	40.8764	82.4267	122.5991	40.1724	82.832	122.4536	39.6216
82.1765	123.0159	40.8394	82.4345	122.6883	40.2538	82.8354	122.509	39.6736
82.1046	123.0017	40.8972	82.3773	122.5705	40.1931	82.7549	122.5636	39.8086
82.2353	122.9965	40.7612	82.3579	122.6697	40.3118	82.6397	122.5965	39.9567
82.2206	122.9939	40.7733	82.3739	122.6276	40.2538	82.6146	122.6796	40.065
82.2102	122.9584	40.7482	82.4917	122.6675	40.1758	82.5403	122.6988	40.1585
82.1661	122.8623	40.6963	82.4908	122.6502	40.1594	82.5479	122.6987	40.1507
82.1453	122.9004	40.7552	82.5505	122.7697	40.2191	82.593	122.6372	40.0442
82.2544	122.9481	40.6937	82.5393	122.7229	40.1836	82.7463	122.6458	39.8996
82.2483	122.884	40.6356	82.5618	122.6328	40.071	82.8259	122.5592	39.7333
82.2839	122.8644	40.5805	82.4969	122.6476	40.1507	82.851	122.6198	39.7688
82.3046	122.9125	40.6079	82.5324	122.6727	40.1403	82.7099	122.6519	39.942
82.2942	122.8736	40.5794	82.5394	122.6803	40.1409	82.4882	122.8069	40.3187

82.5592	122.7705	40.2113	82.5133	122.9091	40.3958	84.5007	121.7539	37.2532
82.5588	122.7957	40.2369	82.425	122.9732	40.5482	84.447	121.7599	37.3129
82.5505	122.7653	40.2148	82.4674	123.0269	40.5594	84.4245	121.8578	37.4333
82.6042	122.6935	40.0892	82.5112	123.1207	40.6095	84.3353	121.908	37.5727
82.5722	122.5497	39.9775	82.6571	123.0589	40.4018	84.447	121.7998	37.3528
82.6034	122.6147	40.0113	82.6796	122.9801	40.3005	84.4703	121.8232	37.3528
82.5549	122.5731	40.0182	82.6155	123.0217	40.4062	84.5596	121.8569	37.2973
82.5938	122.7038	40.11	82.4995	122.9567	40.4573	84.492	121.8673	37.3753
82.696	122.651	39.955	82.6285	122.9203	40.2919	84.6297	121.8223	37.1926
82.6046	122.7305	40.1259	82.6761	122.9342	40.2581	84.5709	121.8916	37.3207
82.6856	122.729	40.0433	82.664	122.8718	40.2079	84.4669	121.8933	37.4264
82.7159	122.7723	40.0563	82.6636	122.9217	40.258	84.3829	122.057	37.674
82.7991	122.748	39.9489	82.6259	122.9411	40.3152	84.6479	121.8846	37.2367
82.7696	122.7671	39.9974	82.6553	122.8831	40.2278	84.4915	122.0046	37.5132
82.7956	122.7238	39.9282	82.6112	122.929	40.3178	84.3119	122.07	37.758
82.7947	122.8649	40.0702	82.5653	122.9446	40.3793	84.1907	122.1635	37.9728
82.8502	122.8346	39.9844	82.5956	122.9489	40.3533	84.15	122.3635	38.2135
82.7041	122.8679	40.1638	82.638	122.9922	40.3542	84.1136	122.3904	38.2767
82.787	122.832	40.0451	82.6146	122.8692	40.2546	84.2045	122.4094	38.2049
82.6519	122.8216	40.1698	82.5852	122.838	40.2528	84.1474	122.4458	38.2984
82.7324	122.8355	40.1031	82.5618	122.8259	40.2641	84.1786	122.4432	38.2646
82.806	122.8173	40.0113	82.5012	122.8511	40.3499	84.1849	122.4389	38.254
82.8441	122.8537	40.0096	82.5012	122.942	40.4408	83.9179	122.49	38.572
82.7844	122.7904	40.0061	82.4094	122.9169	40.5075	83.924	122.5307	38.6067
82.8528	122.7342	39.8814	82.4536	122.8303	40.3767	84.0028	122.5428	38.54
82.8477	122.8151	39.9673	82.4839	122.8459	40.362	84.1846	122.4285	38.2438
82.7956	122.8182	40.0225	82.593	122.6848	40.0918	84.3119	122.4553	38.1434
82.8372	122.8441	40.007	82.6011	122.7895	40.1884	84.1231	122.5237	38.4006
82.8692	122.7974	39.9282	82.5514	122.7835	40.2321	84.0634	122.3895	38.3261
82.9246	122.7064	39.7818	82.4657	122.6069	40.1412	84.0554	122.4548	38.3993
82.8822	122.7506	39.8684	82.5895	122.5627	39.9732	84.0417	122.4536	38.4118
82.9454	122.8121	39.8667	83.5265	122.0977	38.5712	83.9231	122.4891	38.566
82.9688	122.7307	39.7619	83.7525	121.9842	38.2317	83.8278	122.386	38.5582
82.8909	122.7851	39.8942	84.1275	121.8335	37.7061	84.0253	122.4544	38.4292
82.8562	122.8043	39.9481	83.9829	121.8517	37.8689	84.2678	122.3358	38.068
82.8943	122.8485	39.9541	83.9779	121.8188	37.8408	84.2323	122.4891	38.2568
82.987	122.7125	39.7255	83.9976	121.889	37.8914	84.3197	122.4977	38.178
83.0693	122.7272	39.658	84.0288	121.7721	37.7433	84.303	122.5596	38.2566
83.0961	122.7523	39.6562	84.0002	121.7877	37.7875	84.4513	122.5817	38.1304
83.0424	122.7359	39.6935	84.0132	121.7392	37.726	84.5786	122.5895	38.0109
82.9541	122.7203	39.7662	84.0521	121.7054	37.6532	84.5778	122.5445	37.9667
82.8495	122.8212	39.9717	84.3292	121.6266	37.2973	84.6193	122.593	37.9737
82.774	122.8779	40.1039	84.4219	121.4958	37.0739	84.544	122.7108	38.1668
82.6865	122.8667	40.1801	84.4677	121.5096	37.0419	84.2175	122.9212	38.7037
82.6345	122.897	40.2624	84.5258	121.6863	37.1605	84.2756	122.9385	38.663
82.6207	122.9913	40.3707	84.4531	121.6378	37.1848	84.1259	123.0106	38.8847
82.4665	122.8926	40.4261	84.5908	121.7755	37.1848	84.2184	123.0277	38.8093

84.2097	123.0217	38.8119	85.4208	123.1771	37.7563	85.0272	124.0201	38.9929
84.2219	123.0468	38.8249	85.4775	123.1888	37.7113	85.0463	124.0678	39.0215
84.1638	122.9099	38.7461	85.3935	123.2364	37.8429	85.0658	124.0184	38.9525
84.337	122.9377	38.6006	85.5174	123.3031	37.7857	85.106	123.9838	38.8777
84.176	123.045	38.8691	85.5061	123.3377	37.8316	85.1233	123.9716	38.8483
84.1959	123.0017	38.8059	85.5607	123.4607	37.9	85.0194	123.9872	38.9678
84.1699	123.0857	38.9158	85.481	123.4408	37.9598	85.0593	123.9595	38.9002
84.2008	123.0582	38.8574	85.6083	123.4053	37.797	84.9882	124.0669	39.0786
84.266	123.0459	38.7799	85.6776	123.4858	37.8083	84.9882	124.1613	39.173
84.1474	123.2087	39.0613	85.5373	123.5023	37.965	84.9761	124.1448	39.1687
84.1275	123.297	39.1696	85.5156	123.562	38.0464	84.9654	124.0633	39.0979
84.3258	123.2174	38.8916	85.5875	123.5239	37.9364	84.9242	124.137	39.2129
84.4773	123.11	38.6327	85.6022	123.6322	38.0299	84.9484	124.0712	39.1228
84.3137	123.239	38.9254	85.5477	123.5724	38.0247	84.9276	124.195	39.2674
84.2426	123.3403	39.0977	85.6057	123.5923	37.9866	85.0757	124.1483	39.0726
84.0951	123.3568	39.2617	85.6507	123.6798	38.0291	84.9735	124.2106	39.2371
84.1006	123.3594	39.2588	85.3026	123.7257	38.4231	85.0298	124.1803	39.1505
84.2028	122.9481	38.7452	84.9804	123.6968	38.7165	85.0792	124.1907	39.1115
84.1716	122.9827	38.811	85.016	123.6157	38.5998	85.0809	124.1821	39.1012
84.0824	123.0346	38.9522	85.029	123.6348	38.6058	85.0271	124.2853	39.2582
84.0565	123.1429	39.0864	85.2376	123.6114	38.3737	85.0506	124.2591	39.2085
84.0426	123.0918	39.0492	85.3883	123.6348	38.2464	85.093	124.2505	39.1574
83.9058	123.2087	39.3029	85.3009	123.6226	38.3218	85.0593	124.3189	39.2596
83.8238	123.1604	39.3366	85.2169	123.7958	38.579	85.0082	124.2721	39.264
84.0807	123.1897	39.1089	85.184	123.7153	38.5313	85.0887	124.2487	39.16
83.9872	123.2148	39.2276	85.2931	123.7365	38.4434	85.0038	124.3379	39.3341
84.2816	123.1143	38.8327	85.2853	123.7196	38.4344	84.996	124.3457	39.3497
83.7516	123.3048	39.5532	85.3424	123.6859	38.3434	84.9689	124.3188	39.3498
83.9811	123.297	39.3159	85.332	123.7569	38.4248	84.9675	124.3189	39.3514
84.654	123.0442	38.3902	85.3658	123.7101	38.3443	84.9787	124.383	39.4042
84.945	122.9567	38.0118	85.3424	123.7118	38.3694	85.0298	124.3379	39.3081
84.9266	122.9833	38.0567	85.3572	123.8166	38.4595	85.0298	124.389	39.3592
84.7146	123.0632	38.3486	85.3061	123.8738	38.5677	85.0593	124.3492	39.2899
84.8064	123.0121	38.2057	85.2552	123.8589	38.6037	84.99	124.389	39.399
84.8081	123.0805	38.2724	85.2402	123.9405	38.7002	84.8549	124.4756	39.6207
84.9129	123.0953	38.1824	85.1762	123.9266	38.7504	84.8667	124.4967	39.63
84.9008	123.084	38.1832	85.1614	123.8859	38.7245	84.9302	124.4739	39.5437
84.7969	123.0676	38.2707	85.1095	123.9439	38.8344	84.9302	124.5007	39.5705
85.132	123.0788	37.9468	85.0272	123.9491	38.9219	84.9579	124.5648	39.6069
84.9804	123.1005	38.1201	84.9536	123.9855	39.0319	84.8731	124.5925	39.7195
85.0116	123.1559	38.1443	84.9605	123.9387	38.9782	84.7761	124.6731	39.897
85.268	123.0511	37.7831	85.0103	123.955	38.9446	84.8263	124.725	39.8987
85.1493	123.1212	37.9719	84.9857	123.9638	38.9782	84.8159	124.7978	39.9818
85.2299	123.1005	37.8706	84.9779	123.9621	38.9842	84.7619	124.5161	39.7542
85.1995	123.2052	38.0057	84.9883	123.9491	38.9609	84.7579	124.6012	39.8433
85.2325	123.1819	37.9494	85.0948	123.9543	38.8595	84.822	124.5302	39.7082
85.2879	123.1628	37.8749	85.0333	123.9318	38.8985	84.8774	124.4323	39.5549

84.9103	124.4947	39.5844	84.2756	125.0835	40.808	84.377	125.6577	41.2808
84.9701	124.5155	39.5454	83.9006	125.1745	41.2739	84.4505	125.6447	41.1942
84.9276	124.4124	39.4848	83.8235	125.2117	41.3882	84.7224	125.5867	40.8643
84.9432	124.3786	39.4354	83.7352	125.3312	41.596	84.822	125.5598	40.7378
85.0368	124.3778	39.341	83.756	125.3892	41.6332	84.6912	125.5667	40.8755
84.8757	124.4315	39.5558	83.7525	125.4013	41.6488	84.602	125.6196	41.0175
84.9969	124.4834	39.4865	83.7462	125.4181	41.6719	84.1275	125.8949	41.7675
84.6219	124.6549	40.0329	83.7984	125.4187	41.6203	83.6166	125.9651	42.3485
84.7847	124.5345	39.7498	83.7742	125.4343	41.6601	84.4087	125.6542	41.2455
84.9501	124.4748	39.5246	83.7534	125.3823	41.6289	84.0513	125.6551	41.6038
84.9242	124.4505	39.5263	83.9352	125.3373	41.402	84.2297	125.5728	41.3431
84.8739	124.5605	39.6865	83.665	125.4334	41.7683	84.1898	125.552	41.3622
84.8738	124.5064	39.6326	83.6902	125.4559	41.7657	84.3266	125.4715	41.1448
84.8705	124.5562	39.6857	83.8512	125.4031	41.5518	84.5327	125.3693	40.8366
84.9675	124.5492	39.5818	83.8678	125.3186	41.4508	84.5059	125.365	40.8591
84.9138	124.5336	39.6199	83.8737	125.3182	41.4445	84.4903	125.3104	40.8201
84.744	124.6021	39.858	83.9889	125.2758	41.2869	84.3171	125.3732	41.0561
84.8203	124.6124	39.7922	83.9387	125.2411	41.3024	84.4479	125.2974	40.8495
84.8991	124.557	39.658	83.9067	125.255	41.3483	84.279	125.3329	41.0539
84.8393	124.6298	39.7905	83.9188	125.2654	41.3466	84.4158	125.2957	40.8799
84.8384	124.6047	39.7662	84.014	125.2498	41.2358	84.2859	125.3312	41.0453
84.6641	124.7539	40.0898	84.0833	125.2186	41.1353	84.2842	125.4135	41.1293
84.5838	124.7787	40.1949	83.9136	125.2498	41.3362	84.2574	125.4187	41.1613
84.7172	124.6843	39.9671	83.9154	125.2587	41.3433	84.3076	125.4117	41.1041
84.6349	124.7493	40.1143	84.1024	125.2455	41.1431	84.488	125.3547	40.8667
84.3396	124.9025	40.5629	84.0885	125.2463	41.1578	84.3007	125.4438	41.1431
84.1231	125.029	40.9058	84.1413	125.2299	41.0886	84.2903	125.4213	41.131
84.2089	124.9701	40.7612	84.2487	125.2723	41.0236	84.1266	125.475	41.3483
84.1257	125.048	40.9223	84.2219	125.2671	41.0453	84.0184	125.5408	41.5224
84.3453	124.9794	40.6342	84.1604	125.3009	41.1405	84.1231	125.4966	41.3735
84.3007	124.8904	40.5898	84.2764	125.3165	41.0401	84.1249	125.4698	41.3449
84.1266	124.9857	40.8591	84.2765	125.3001	41.0235	83.9837	125.5468	41.5631
84.0236	125.0134	40.9898	84.344	125.3139	40.9699	84.1102	125.5382	41.428
84.1335	125.0272	40.8937	84.0374	125.3987	41.3613	84.0449	125.5723	41.5274
84.111	125.0359	40.9249	84.1734	125.4213	41.2479	84.0305	125.5659	41.5354
84.0166	125.0905	41.0738	84.1353	125.4646	41.3293	83.9777	125.5936	41.6159
84.04	125.0913	41.0513	84.2981	125.5027	41.2046	83.8798	125.6378	41.7579
84.0361	125.0437	41.0077	84.1812	125.5971	41.4159	83.8616	125.6715	41.8099
84.3916	124.9519	40.5603	84.2167	125.6888	41.4722	83.8296	125.694	41.8645
84.35	124.9813	40.6313	84.2246	125.7079	41.4834	83.8088	125.7044	41.8956
83.9413	125.139	41.1977	84.2686	125.6862	41.4176	83.911	125.7079	41.7969
84.1396	125.1329	40.9933	84.1188	125.72	41.6012	83.9806	125.6912	41.7106
84.4938	124.9406	40.4469	84.1197	125.7356	41.6159	83.9162	125.7001	41.7839
84.0383	125.0974	41.0591	83.9993	125.7668	41.7675	83.8469	125.7555	41.9086
83.859	125.1597	41.3007	84.0495	125.7832	41.7337	83.8876	125.746	41.8584
83.6907	125.2992	41.6085	84.0504	125.7971	41.7467	83.7205	125.8066	42.0861
83.9855	125.2074	41.2219	84.8506	125.5338	40.6833	83.7794	125.8439	42.0645

83.8486	125.8629	42.0143	83.3775	126.3782	43.0006	83.6711	125.9062	42.2351
83.9344	125.862	41.9277	83.3923	126.334	42.9417	83.7101	125.8568	42.1468
83.9955	125.8938	41.8983	83.381	126.295	42.914	83.6538	125.8456	42.1918
84.0227	125.8846	41.8619	83.517	126.2188	42.7019	83.6425	125.8378	42.1953
83.9681	125.9209	41.9528	83.2979	126.2479	42.9501	83.5516	125.8222	42.2706
83.9196	125.9504	42.0307	83.2528	126.2838	43.0309	83.5421	125.8257	42.2836
83.9456	125.9582	42.0125	83.3256	126.2457	42.9201	83.6863	125.8163	42.13
83.9335	125.9538	42.0203	83.3005	126.1824	42.882	83.7222	125.7555	42.0333
83.8937	125.9668	42.0732	83.226	126.1651	42.9391	83.7231	125.7685	42.0454
83.833	126.043	42.21	83.1983	126.1366	42.9383	83.7023	125.7148	42.0125
83.8255	126.0647	42.2392	83.0736	126.1305	43.0569	83.7222	125.688	41.9658
83.8997	126.0318	42.132	83.0467	126.1478	43.1011	83.7231	125.7079	41.9848
83.8486	126.0915	42.2429	83.0195	126.1484	43.1289	83.7014	125.7356	42.0342
83.8192	126.1392	42.32	82.9827	126.1443	43.1617	83.6858	125.7763	42.0905
83.8885	126.1158	42.2273	82.9913	126.1236	43.1322	83.6802	125.7397	42.0595
83.9707	126.0794	42.1087	83.1732	126.0811	42.908	83.633	125.7529	42.1199
84.0028	126.1132	42.1104	83.058	126.0638	43.0058	83.6642	125.7581	42.0939
84.0695	126.0716	42.0022	83.1255	126.1625	43.037	83.7309	125.7122	41.9814
84.0537	126.0762	42.0225	83.0407	126.2465	43.2058	83.8304	125.6871	41.8567
84.0504	125.9001	41.8497	82.9905	126.2491	43.2587	83.8088	125.6837	41.8749
83.9474	125.8447	41.8974	83.0671	126.2321	43.165	83.8746	125.7001	41.8255
83.9292	125.8785	41.9493	82.9342	126.2587	43.3245	83.9153	125.7356	41.8203
83.8235	125.9296	42.1061	83.0338	126.2916	43.2578	83.8079	125.7229	41.915
83.8149	126.0196	42.2048	83.0615	126.3253	43.2639	83.8296	125.7607	41.9311
83.7594	126.0361	42.2767	83.084	126.4691	43.3851	83.7949	125.7936	41.9987
83.6841	126.1296	42.4455	83.0459	126.5228	43.4769	83.8114	125.8361	42.0247
83.7181	126.2012	42.4832	83.1004	126.5843	43.4838	84.0175	125.7824	41.7649
83.6988	126.211	42.5122	83.3905	126.463	43.0725	84.0798	125.7746	41.6947
83.6599	126.2154	42.5555	83.4274	126.3387	42.9113	84.1595	125.772	41.6125
83.6408	126.2682	42.6274	83.4105	126.3063	42.8958	84.0513	125.8248	41.7735
83.6599	126.2812	42.6213	83.4208	126.2742	42.8534	84.3021	125.7802	41.4781
83.6451	126.2552	42.6101	83.4226	126.2283	42.8058	84.3015	125.8023	41.5008
83.6529	126.2465	42.5936	83.336	126.1972	42.8612	84.3847	125.7962	41.4116
83.5914	126.2846	42.6932	83.3195	126.2128	42.8932	84.5102	125.7945	41.2843
83.5672	126.3271	42.7599	83.4373	126.1513	42.714	84.5856	125.8153	41.2297
83.8819	126.2497	42.3678	83.5256	126.1807	42.6551	84.6869	125.7677	41.0808
84.0729	126.1409	42.068	83.588	126.1435	42.5555	84.6315	125.7763	41.1448
84.0963	126.1504	42.0541	83.5348	126.1634	42.6285	84.6384	125.8846	41.2462
83.9976	126.1452	42.1476	83.5516	126.1859	42.6343	84.7311	125.9686	41.2375
83.9707	126.1236	42.1528	83.4719	126.2136	42.7417	84.7117	125.9995	41.2878
83.8686	126.1686	42.3	83.4399	126.2024	42.7625	84.6999	126.0534	41.3535
83.8737	126.1833	42.3096	83.5646	126.1608	42.5962	84.7085	126.1045	41.396
83.769	126.2431	42.4741	83.6547	126.1565	42.5018	84.6523	126.1322	41.48
83.6502	126.3334	42.6832	83.73	126.0448	42.3148	84.66	126.1591	41.499
83.5005	126.431	42.9305	83.6971	126.0049	42.3078	84.6306	126.1937	41.5631
83.2961	126.4327	43.1366	83.704	125.952	42.248	84.7302	126.2716	41.5415
83.4122	126.4041	42.992	83.7118	125.901	42.1892	84.6704	126.2751	41.6047

84.8421	126.2215	41.3794	85.1805	127.1523	41.9718	85.655	128.6548	42.9997
84.9415	126.2595	41.318	85.3883	127.1264	41.738	85.6828	128.6981	43.0153
84.9198	126.3071	41.3873	85.3345	127.1394	41.8049	85.6429	128.7648	43.1218
84.9025	126.3383	41.4358	85.3199	127.1194	41.7995	85.5771	128.8141	43.237
84.8306	126.3877	41.557	85.3745	127.1549	41.7805	85.4992	128.8589	43.3597
84.835	126.4137	41.5787	85.487	127.148	41.661	85.3684	128.8609	43.4925
84.7259	126.4422	41.7164	85.3753	127.2259	41.8506	85.2809	128.9492	43.6683
84.7181	126.5297	41.8116	85.3286	127.2528	41.9242	85.2827	128.9241	43.6414
84.7214	126.5545	41.8331	85.4515	127.2181	41.7666	85.2498	128.9085	43.6588
84.751	126.6726	41.9216	85.5321	127.245	41.7129	85.2957	128.9042	43.6085
84.9527	126.6388	41.6861	85.5327	127.2072	41.6745	85.3857	128.8903	43.5046
84.8566	126.696	41.8393	85.4845	127.3004	41.816	85.5113	128.8661	43.3548
84.8107	126.7687	41.958	85.4446	127.3671	41.9225	85.3979	128.9338	43.5359
84.9917	126.7609	41.7692	85.203	127.5905	42.3875	85.5702	128.8349	43.2647
84.9605	126.793	41.8324	85.1978	127.6459	42.4481	85.7685	128.8271	43.0586
84.9631	126.8466	41.8835	85.2446	127.6563	42.4118	85.7616	128.828	43.0664
84.887	126.8381	41.9511	85.2013	127.7022	42.5009	85.688	128.8626	43.1747
84.822	126.7774	41.9554	85.061	127.7559	42.6949	85.5338	128.9051	43.3713
84.7466	126.7938	42.0472	84.917	127.8406	42.9236	85.4403	128.9458	43.5055
84.7778	126.8406	42.0628	84.8393	127.8893	43.05	85.6828	128.8981	43.2154
84.8471	126.9047	42.0576	84.9908	127.8434	42.8525	85.6287	128.9435	43.3148
84.6479	126.7722	42.1243	85.0411	127.8018	42.7607	85.8161	128.8748	43.0586
84.7614	126.8302	42.0688	85.1138	127.7862	42.6724	85.8023	128.8895	43.0872
84.7804	126.8501	42.0697	85.1155	127.8217	42.7062	85.8672	128.8592	42.992
84.8315	126.8945	42.063	85.0359	127.846	42.8101	85.9365	128.8462	42.9097
84.8558	126.9739	42.1182	85.0593	127.8841	42.8248	86.0309	128.802	42.7711
84.8341	127.0034	42.1693	85.1231	127.8468	42.7237	86.0759	128.7405	42.6646
84.9969	126.9384	41.9415	85.1441	127.8313	42.6871	86.1244	128.7656	42.6412
84.9848	126.9246	41.9398	85.1346	127.8529	42.7183	86.13	128.814	42.684
84.9501	126.9904	42.0403	85.2307	127.8243	42.5936	86.1435	128.8193	42.6759
84.9363	127.0372	42.1009	85.19	127.8183	42.6282	86.0724	128.8427	42.7703
84.8696	127.1593	42.2897	85.2273	127.7888	42.5616	86.0854	128.8661	42.7807
84.9725	127.1923	42.2198	85.2472	127.8018	42.5546	86.0534	128.8869	42.8335
85.0532	127.0155	41.9623	85.1104	127.8607	42.7503	86.0378	128.8661	42.8283
84.9415	127.0389	42.0974	85.131	127.9164	42.7853	86.1339	128.8531	42.7192
84.8436	127.0839	42.2403	85.132	127.9196	42.7876	86.1123	128.8003	42.688
84.7995	127.1506	42.3511	85.1173	127.9404	42.8231	86.085	128.792	42.7069
84.7544	127.1991	42.4447	85.1502	127.9075	42.7573	86.0785	128.8037	42.7252
84.6271	127.2181	42.591	85.1805	127.8789	42.6984	86.1937	128.7942	42.6005
84.5734	127.2233	42.6499	85.2411	127.8451	42.604	86.14	128.8063	42.6663
84.5549	127.2345	42.6796	85.184	127.8598	42.6759	86.0586	128.8029	42.7443
84.7042	127.2156	42.5113	85.2558	127.8676	42.6118	85.9945	128.8254	42.8309
84.8168	127.2138	42.397	85.3371	127.8406	42.5035	86.0075	128.8453	42.8378
85.016	127.1774	42.1615	85.3831	127.8685	42.4854	86.0153	128.8557	42.8404
84.8159	127.1645	42.3485	85.3216	127.9031	42.5815	86.0621	128.8184	42.7563
84.8791	127.1965	42.3174	85.4879	128.6591	43.1712	86.0785	128.8392	42.7607
85.048	127.1852	42.1372	85.578	128.6669	43.0889	86.011	128.8306	42.8196

85.9815	128.8418	42.8603	86.4362	129.2627	42.8266	86.9298	128.6383	41.7086
86.0785	128.7856	42.707	86.4474	129.2411	42.7936	86.9518	128.6308	41.6789
86.0647	128.8081	42.7434	86.4682	129.254	42.7859	86.9635	128.6591	41.6956
86.0802	128.8176	42.7374	86.4491	129.2255	42.7763	86.8977	128.7007	41.803
86.1755	128.8271	42.6516	86.482	129.2644	42.7824	86.8449	128.7206	41.8757
86.3392	128.815	42.4758	86.489	129.2696	42.7807	86.7297	128.8193	42.0896
86.3581	128.8122	42.4541	86.5617	129.2506	42.6889	86.747	128.8375	42.0905
86.3002	128.8332	42.533	86.5854	129.2633	42.6779	86.7375	128.8488	42.1113
86.2881	128.8505	42.5624	86.65	129.2393	42.5893	86.7072	128.9051	42.1979
86.185	128.912	42.727	86.6301	129.254	42.6239	86.6726	128.9162	42.2436
86.1045	128.9284	42.824	86.5522	129.3458	42.7936	86.7444	128.8869	42.1424
86.0075	128.9951	42.9876	86.5851	129.3536	42.7685	86.734	128.9362	42.2022
86.0439	128.9761	42.9322	86.5427	129.364	42.8214	86.6145	128.9882	42.3737
86.0265	128.9761	42.9495	86.5072	129.4047	42.8976	86.6388	128.9778	42.339
85.9758	128.9734	42.9976	86.4907	129.3995	42.9088	86.6016	128.9908	42.3892
85.9529	128.9778	43.0249	86.4214	129.4463	43.0249	86.5938	129.0878	42.494
85.972	128.9891	43.0171	86.5889	129.4218	42.8329	86.6448	129.1389	42.494
86.0508	128.9605	42.9097	86.6907	129.3874	42.6967	86.592	129.1597	42.5676
86.1322	128.9388	42.8066	86.786	129.3727	42.5867	86.6039	129.1866	42.5827
86.2898	128.8955	42.6057	86.7349	129.3311	42.5962	86.6474	129.2411	42.5936
86.3409	128.8739	42.533	86.7652	129.3606	42.5953	86.6319	129.3545	42.7226
86.3565	128.854	42.4975	86.7739	129.3441	42.5702	86.6431	129.4879	42.8447
86.2594	128.8959	42.6365	86.7626	129.3372	42.5746	86.5487	129.5762	43.0275
86.1911	128.9267	42.7356	86.7193	129.4143	42.6949	86.6293	129.6429	43.0136
86.1885	128.9336	42.7452	86.6638	129.4368	42.773	86.6024	129.7442	43.1418
86.1538	128.9639	42.8101	86.5219	129.4688	42.9469	86.5505	129.9312	43.3808
86.0802	129.0228	42.9426	86.3478	129.5381	43.1903	86.5634	129.709	43.1456
85.9893	129.086	43.0967	86.2439	129.5996	43.3557	86.5955	129.9208	43.3254
85.8967	129.1337	43.237	86.0075	129.7702	43.7627	86.76	129.9347	43.1747
85.8906	129.1597	43.2691	85.9755	129.8083	43.8328	86.7765	129.9191	43.1426
85.9732	129.1549	43.1817	86.0603	129.7485	43.6882	87.0813	129.8126	42.7313
85.9841	129.1458	43.1617	86.2465	129.7009	43.4544	87.1558	129.7771	42.6213
85.9997	129.164	43.1643	87.2214	128.8052	41.5838	87.2043	129.7381	42.5339
85.959	129.2194	43.2604	88.3967	128.4972	40.1005	87.1783	129.7442	42.5659
86.0179	129.2341	43.2162	87.665	128.3803	40.7153	87.217	129.7169	42.4999
85.9477	129.2904	43.3427	87.2251	129.1917	41.9666	87.1973	129.7416	42.5442
85.7096	129.3692	43.6596	87.0778	128.7847	41.7069	87.2588	129.6723	42.4135
85.6429	129.4229	43.78	87.0726	128.7102	41.6376	87.3489	129.6992	42.3503
85.797	129.3839	43.587	87.154	128.6357	41.4817	87.2935	129.7225	42.4291
85.8733	129.3779	43.5046	87.1281	128.6661	41.538	87.2952	129.7295	42.4343
85.8447	129.4194	43.5748	87.1245	128.5858	41.4614	87.3775	129.6879	42.3104
85.9651	129.3744	43.4094	86.954	128.6894	41.7354	87.3541	129.7433	42.3892
86.1114	129.3484	43.237	86.8241	128.6565	41.8324	87.3077	129.8015	42.4938
86.263	129.3008	43.0379	86.7964	128.6539	41.8575	87.2199	129.8273	42.6075
86.4604	129.2047	42.7443	86.9029	128.6808	41.7779	87.2268	129.8412	42.6144
86.4405	129.222	42.7815	86.9341	128.6609	41.7268	87.1662	129.8741	42.7079
86.448	129.2562	42.8082	86.9427	128.6738	41.7311	87.1887	129.8576	42.6689

87.2839	129.8827	42.5988	87.1133	130.469	43.3557	88.511	129.8334	41.3224
87.2138	129.8931	42.6793	87.1662	130.4638	43.2976	88.2919	129.946	41.654
87.1411	129.8923	42.7512	87.0926	130.5045	43.4119	88.2382	129.9633	41.725
87.0857	129.8922	42.8065	87.1177	130.4915	43.3738	88.4262	129.9356	41.5094
87.0475	129.8983	42.8508	87.0683	130.4855	43.4171	88.3898	129.9321	41.5423
87.0224	129.9243	42.9019	87.1289	130.4807	43.3518	88.6989	129.7494	41.0504
87.0709	129.9027	42.8317	87.1887	130.4829	43.2942	88.7717	129.7199	40.9483
87.019	129.9139	42.895	87.1921	130.4733	43.2812	88.6581	129.7698	41.1116
86.9791	129.9027	42.9235	87.1679	130.4474	43.2795	88.3387	129.9979	41.6592
86.9999	129.9243	42.9244	87.1973	130.4889	43.2916	88.6452	129.8949	41.2496
87.0172	129.9295	42.9123	87.1947	130.5071	43.3124	88.4634	130.01	41.5467
87.0708	129.9407	42.8699	87.1705	130.514	43.3435	87.5619	130.3833	42.8214
87.0804	129.9598	42.8794	87.1333	130.4993	43.3661	87.8901	130.269	42.3788
87.0822	129.9823	42.9002	87.5394	130.2543	42.7149	88.085	130.2222	42.1372
87.1506	130.0083	42.8577	87.762	130.2023	42.4403	88.6548	130.0533	41.3986
87.1939	129.9841	42.7902	88.4868	129.8091	41.3224	88.4652	130.2032	41.738
87.2077	130.0326	42.8248	88.5301	129.6368	41.1067	88.4114	130.2949	41.8835
87.2493	130.049	42.7997	88.4565	129.6714	41.215	88.9241	130.0975	41.1734
87.2103	130.0836	42.8733	88.4045	129.7	41.2955	89.3692	129.8879	40.5187
87.0674	130.1685	43.1011	88.6158	129.5736	40.9578	89.3077	129.92	40.6123
87.0822	130.1794	43.0972	88.7206	129.5164	40.7959	88.9873	130.036	41.0487
87.0285	130.2335	43.205	88.6426	129.5753	40.9327	88.8877	130.1157	41.228
87.0397	130.2387	43.1989	88.5375	129.6235	41.0861	88.8661	130.1183	41.2522
87.0882	130.2222	43.134	88.5422	129.6662	41.1241	88.8946	130.1798	41.2851
86.9834	130.2724	43.289	88.582	129.6039	41.0219	88.4582	130.3794	41.9212
86.9168	130.3374	43.4206	88.5881	129.6758	41.0877	88.7284	130.185	41.4566
86.9375	130.3183	43.3808	88.4998	129.713	41.2133	88.9795	130.0845	41.105
86.8432	130.3668	43.5237	88.5327	129.6983	41.1656	88.64	130.2776	41.6376
86.8505	130.4031	43.5526	88.5214	129.6749	41.1535	88.6851	130.2352	41.5501
86.8899	130.3677	43.4778	88.5846	129.7043	41.1197	89.1224	130.0646	40.9422
86.9514	130.3885	43.4371	88.6493	129.6359	40.9865	89.041	130.0481	41.0071
86.941	130.3893	43.4483	88.5586	129.7234	41.1648	88.9856	130.075	41.0894
86.9826	130.3486	43.3661	88.5292	129.8117	41.2825	88.8713	130.0904	41.2191
87.2008	130.2534	43.0526	88.5119	129.8663	41.3544	88.8678	130.0741	41.2063
87.1402	130.3296	43.1894	88.5249	129.8576	41.3328	88.4192	130.3027	41.8835
87.0726	130.3694	43.2968	88.5067	129.8871	41.3804	88.4019	130.366	41.964
87.0285	130.4084	43.38	88.55	129.8992	41.3492	88.7292	130.2205	41.4912
87.051	130.4326	43.3816	88.3967	129.9044	41.5077	88.6045	130.2646	41.6601
87.0796	130.4179	43.3383	88.3287	129.8816	41.553	88.3759	130.3582	41.9822
87.2718	130.3504	43.0786	88.4201	129.8169	41.3968	88.6903	130.2075	41.5172
87.2493	130.3019	43.0526	88.5405	129.7589	41.2184	88.5912	130.2657	41.6745
87.1653	130.359	43.1937	88.5552	129.7494	41.1942	88.7318	130.2092	41.4774
87.1627	130.3937	43.231	88.5543	129.7797	41.2254	88.5032	130.3201	41.8168
87.1947	130.3668	43.1721	88.6063	129.7433	41.137	88.44	130.3911	41.9511
87.1263	130.4155	43.2892	88.5751	129.7684	41.1933	88.9423	130.1945	41.2522
87.0822	130.4534	43.3712	88.2833	129.9226	41.6393	88.9111	130.1304	41.2193
87.1185	130.4586	43.3401	88.4309	129.8464	41.4155	88.6807	130.2213	41.5406

88.4504	130.3434	41.893	87.0302	131.1098	44.0796	87.3879	131.0345	43.6466
88.3296	130.3776	42.048	87.1835	131.0362	43.8527	87.3749	131.0405	43.6657
88.5257	130.3434	41.8177	87.3956	130.9678	43.5722	87.3697	131.0769	43.7072
88.8617	130.1867	41.325	87.5042	130.8718	43.3676	87.4433	131.0492	43.6059
88.9934	130.0793	41.086	87.561	130.876	43.315	87.4727	131.076	43.6033
88.6989	130.2785	41.5796	87.5472	130.8864	43.3392	87.409	131.1237	43.7147
88.4876	130.3417	41.8541	87.5134	130.8821	43.3687	87.5316	131.089	43.5574
88.3621	130.4023	42.0403	87.5446	130.8725	43.328	87.6909	131.0596	43.3687
87.8841	130.6006	42.7166	87.6476	130.8881	43.2405	87.6269	131.0717	43.4449
88.1798	130.5053	42.3255	87.555	130.8994	43.3444	87.7031	131.0449	43.3418
88.072	130.5634	42.4914	87.5645	130.9003	43.3357	87.7585	131.0518	43.2933
88.0287	130.5842	42.5555	87.4716	130.929	43.4575	87.8009	131.0674	43.2665
88.1473	130.566	42.4187	87.4415	130.9462	43.5046	88.1906	130.8907	42.7001
87.9655	130.6032	42.6378	87.4623	130.9678	43.5055	89.2387	130.3415	41.1028
88.1031	130.5305	42.4273	87.3679	130.9747	43.6068	89.4333	130.2897	40.8565
87.7723	130.6993	42.927	87.3601	130.9756	43.6155	89.4125	130.3001	40.8876
87.9317	130.6483	42.7166	87.3775	130.9643	43.5869	89.448	130.3071	40.8591
87.8856	130.6815	42.7959	87.3757	130.9539	43.5782	89.6091	130.2387	40.6296
87.7264	130.7262	42.9997	87.3342	130.9496	43.6155	89.532	130.2473	40.7153
87.4996	130.7764	43.2769	87.3209	130.9423	43.6213	89.4965	130.2733	40.7768
87.6953	130.7323	43.037	87.29	130.9825	43.6925	89.4212	130.2707	40.8495
87.7221	130.6993	42.9772	87.2701	130.9513	43.6813	89.4527	130.3045	40.8518
87.6762	130.721	43.0448	87.245	130.986	43.741	89.4402	130.3278	40.8876
87.6104	130.7903	43.1799	87.2632	130.9297	43.6665	89.4142	130.3313	40.9171
87.4857	130.8734	43.3877	87.251	130.9314	43.6804	89.4696	130.2897	40.8201
87.3509	130.9238	43.5729	87.2701	131.0276	43.7575	89.5294	130.2534	40.724
87.2467	130.9652	43.7185	87.2891	130.9643	43.6752	89.5692	130.2101	40.6408
87.3584	130.9375	43.5791	87.3456	130.929	43.5834	89.4809	130.2811	40.8002
87.4312	130.9513	43.5202	87.4095	130.9817	43.5722	89.5251	130.2681	40.743
87.4935	130.9054	43.4119	87.2978	131.0232	43.7254	89.4712	130.2772	40.806
87.5229	130.8821	43.3591	87.2103	131.0475	43.8371	89.4307	130.2837	40.853
87.6017	130.8751	43.2734	87.1064	131.0856	43.9792	89.3969	130.3183	40.9214
87.5195	130.9011	43.3816	87.2008	131.0631	43.8623	89.4766	130.2915	40.8149
87.5732	130.863	43.2898	87.303	131.0128	43.7098	89.5459	130.2707	40.7248
87.3967	130.9282	43.5315	87.3705	130.9825	43.612	89.558	130.2629	40.7049
87.4762	130.9063	43.4301	87.3749	130.9877	43.6129	89.5545	130.2785	40.724
87.5013	130.9003	43.399	87.3456	131.0171	43.6715	89.5424	130.2742	40.7318
87.5377	130.8942	43.3565	87.3151	130.9877	43.6726	89.4835	130.2923	40.8088
87.4372	130.8673	43.4301	87.2865	131.0327	43.7462	89.5531	130.2587	40.7055
87.645	130.85	43.205	87.2216	131.083	43.8614	89.5251	130.3027	40.7777
87.6953	130.8232	43.1279	87.193	131.0353	43.8423	89.4818	130.3521	40.8703
87.6199	130.8249	43.205	87.1783	131.0769	43.8986	89.4766	130.3296	40.853
87.5931	130.8462	43.2531	87.2718	131.0942	43.8224	89.5164	130.2828	40.7664
87.4156	130.9479	43.5323	87.3965	131.0483	43.6518	89.5606	130.2196	40.659
87.3515	130.9912	43.6397	87.5799	130.944	43.3641	89.6749	130.1945	40.5196
87.1263	131.089	43.9627	87.5463	130.9522	43.4059	89.6099	130.2438	40.6339
86.9627	131.1445	44.1818	87.3714	131.0362	43.6648	89.7346	130.1891	40.4545

89.7164	130.1235	40.407	89.9736	129.7303	39.7567	82.7203	123.0156	40.2953
89.6593	130.1547	40.4954	90.0218	129.7389	39.7172	82.7185	122.9723	40.2538
89.5883	130.2283	40.64	90.0239	129.7511	39.7272	82.6882	122.9957	40.3074
89.5917	130.2352	40.6434	89.9788	129.7935	39.8147	82.6787	123.0295	40.3507
89.674	130.178	40.504	90.0048	129.7658	39.761	82.7099	122.9801	40.2702
89.655	130.1642	40.5092	89.9962	129.7546	39.7584	82.9056	122.9688	40.0632
89.668	130.1798	40.5118	89.9736	129.7814	39.8078	82.8926	122.9515	40.0589
89.6677	130.1759	40.5082	90.0135	129.7546	39.7411	82.8539	122.978	40.1241
89.6376	130.1486	40.511	90.0195	129.7702	39.7506	82.8259	122.955	40.1291
89.6082	130.185	40.5768	89.894	129.9231	40.029	82.8173	122.9584	40.1412
89.6342	130.1702	40.5361	89.8697	129.9607	40.091	82.7514	122.9541	40.2027
89.6013	130.1997	40.5984	89.9355	129.8325	39.897	82.78	122.9368	40.1568
89.5649	130.2482	40.6833	89.9555	129.8291	39.8736	82.7514	122.9238	40.1724
89.6143	130.2646	40.6504	89.9373	129.8888	39.9515	82.6865	123.0182	40.3317
89.6125	130.2343	40.6218	89.9295	129.8905	39.9611	82.6709	122.9939	40.323
89.6879	130.1477	40.4598	89.9632	129.9624	39.9992	82.6934	122.9888	40.2953
89.713	130.1226	40.4096	89.997	129.9945	39.9974	82.7385	122.9181	40.1796
89.7052	130.1988	40.4936	89.9909	129.9653	39.9744	82.7896	122.884	40.0944
89.7416	130.2404	40.4988	90.0031	129.9823	39.9792	82.8467	122.8831	40.0364
89.6723	130.2603	40.588	89.9355	130.0447	40.1091	82.8051	122.8718	40.0667
89.6394	130.249	40.6097	89.9425	130.0542	40.1117	82.7887	122.8623	40.0736
89.5935	130.288	40.6945	89.8966	130.0784	40.1819	82.8095	122.8632	40.0537
89.5961	130.2672	40.6712	89.9078	130.03	40.1221	82.7982	122.8727	40.0745
89.5461	130.2728	40.7267	89.7312	130.0871	40.3559	82.7255	122.9437	40.2183
89.5917	130.2828	40.6911	89.8576	130.0369	40.1793	82.6434	122.9895	40.3461
89.6498	130.2854	40.6356	90.0103	129.9477	39.9374	82.5583	123.0381	40.4798
89.5848	129.6844	40.0996	90.0057	129.9832	39.9775	82.5202	123.0277	40.5075
89.474	128.3058	38.8318	82.7393	122.8138	40.0745	82.5384	123.0346	40.4962
89.5051	128.6903	39.1851	82.838	122.658	39.8199	82.5168	123.0866	40.5698
89.4956	129.8169	40.3213	82.832	122.7956	39.9637	82.6103	123.0485	40.4382
89.4021	129.9901	40.588	82.8865	122.52	39.6335	82.7584	123.0043	40.246
89.7337	129.8957	40.162	82.8822	122.6528	39.7705	82.7437	122.9939	40.2503
89.8784	129.8048	39.9264	82.8614	122.6502	39.7887	82.7279	123.0318	40.3038
89.8065	129.8723	40.0658	82.9896	122.6813	39.6917	82.7203	123.0771	40.3568
89.9174	129.8204	39.903	83.0719	122.6987	39.6268	82.6796	123.1186	40.4391
89.8844	129.8481	39.9637	83.2399	122.677	39.4371	82.6675	123.0831	40.4157
89.8221	129.8602	40.0381	83.3429	122.6805	39.3376	82.6302	123.1299	40.4997
89.913	129.8221	39.9091	83.2468	122.7775	39.5307	82.6605	123.0814	40.4209
89.9217	129.7944	39.8727	83.1754	122.7966	39.6212	82.6649	123.0693	40.4044
89.9026	129.7883	39.8857	83.0104	122.8606	39.8502	82.7653	123.0269	40.2615
90.0244	129.7275	39.7031	82.9168	122.9082	39.9914	82.594	123.0908	40.4968
89.9598	129.7806	39.8208	82.7852	122.8926	40.1074	82.7272	123.0606	40.3334
89.9503	129.7606	39.8104	82.7125	122.9333	40.2208	82.722	123.0502	40.3282
89.9927	129.7641	39.7714	82.7159	122.9411	40.2252	82.7099	123.0554	40.3455
89.9364	129.778	39.8416	82.6943	122.9671	40.2728	82.7341	123.0676	40.3334
89.9754	129.7589	39.7835	82.6943	122.9515	40.2572	82.7082	123.0727	40.3646
89.9892	129.7165	39.7272	82.6777	122.986	40.3083	82.6527	123.1273	40.4746

82.664	123.0814	40.4174	82.6865	123.2425	40.556	82.664	123.3966	40.7326
82.6883	123.1348	40.4466	82.6675	123.2555	40.588	82.6649	123.394	40.7292
82.7991	123.1126	40.3135	82.6943	123.2433	40.5491	82.6345	123.401	40.7664
82.8961	123.0667	40.1706	82.7323	123.2441	40.5117	82.6034	123.4113	40.808
82.8787	123.1256	40.2468	82.7454	123.2356	40.4902	82.6432	123.4217	40.7785
82.9558	123.0546	40.0987	82.7549	123.2459	40.491	82.565	123.4273	40.8623
82.9359	123.0494	40.1135	82.5869	123.2659	40.6789	82.4847	123.4512	40.9664
82.8935	123.0866	40.1931	82.5367	123.3161	40.7794	82.6293	123.4469	40.8175
82.8303	123.0831	40.2529	82.5142	123.3499	40.8357	82.6605	123.4408	40.7803
82.8231	123.0652	40.2422	82.4839	123.3473	40.8634	83.0095	123.3525	40.343
82.8112	123.084	40.2728	82.5479	123.349	40.801	83.1654	123.336	40.1706
82.6735	123.1325	40.459	82.4954	123.3436	40.8482	83.3845	123.2546	39.8701
82.6094	123.1533	40.5439	82.4388	123.3325	40.8937	83.5265	123.0416	39.5151
82.4795	123.1975	40.7179	82.4605	123.3412	40.8807	83.8493	122.882	39.0327
82.4873	123.1983	40.711	82.4414	123.3802	40.9387	83.8547	122.832	38.9773
82.4267	123.2269	40.8002	82.4181	123.3568	40.9387	83.9422	122.8069	38.8647
82.4691	123.226	40.7569	82.3791	123.3819	41.0028	83.7594	122.8918	39.1323
82.4709	123.2373	40.7664	82.4267	123.3758	40.9491	83.9058	122.8563	38.9505
82.5685	123.1974	40.6289	82.4258	123.381	40.9552	84.0322	122.8104	38.7781
82.5748	123.1983	40.6235	82.4328	123.3966	40.9639	83.937	122.8545	38.9176
82.69	123.1697	40.4798	82.4742	123.4106	40.9363	83.8521	122.8831	39.031
82.4821	123.233	40.7508	82.4605	123.3984	40.9379	83.827	122.8805	39.0535
82.5228	123.1949	40.672	82.554	123.4105	40.8565	83.8431	122.8362	38.9931
82.5999	123.168	40.5681	82.5731	123.3984	40.8253	83.9785	122.8658	38.8873
82.6649	123.1152	40.4503	82.5765	123.3966	40.8201	83.8634	122.9203	39.057
82.5904	123.1923	40.6019	82.5748	123.3984	40.8236	83.885	122.9039	39.0189
82.6002	123.1947	40.5945	82.6432	123.368	40.7248	83.6728	123.0017	39.3289
82.6415	123.168	40.5265	82.5947	123.3507	40.756	83.6555	123.0113	39.3557
82.4077	123.6071	41.1994	82.6927	123.3172	40.6245	83.5871	123.0502	39.4631
82.6198	123.2052	40.5854	82.6553	123.3308	40.6755	83.743	122.9922	39.2492
82.7047	123.1853	40.4806	82.6709	123.3343	40.6634	83.7753	123.0001	39.2247
82.8337	123.1628	40.3291	82.5618	123.3473	40.7855	83.7369	122.9654	39.2284
82.8684	123.1715	40.3031	82.5324	123.3888	40.8565	83.6971	123.013	39.3159
82.8588	123.1533	40.2945	82.4362	123.4252	40.989	83.7118	123.0398	39.328
82.8556	123.1604	40.3047	82.4388	123.4477	41.0089	83.5862	123.0753	39.4891
82.7809	123.181	40.4001	82.4094	123.4798	41.0704	83.6876	123.0277	39.3402
82.6874	123.213	40.5257	82.5244	123.3947	40.8703	83.53	123.0927	39.5627
82.6752	123.2572	40.582	82.5453	123.3784	40.8331	83.5248	123.1394	39.6147
82.5973	123.2494	40.6521	82.5479	123.3992	40.8513	83.5207	123.1331	39.6123
82.5835	123.2858	40.7023	82.6761	123.3932	40.717	83.7421	123.1083	39.3661
82.6605	123.2589	40.5984	82.6934	123.3854	40.6919	84.3838	122.968	38.5842
82.6164	123.2607	40.6443	82.6709	123.3906	40.7196	83.6936	123.1126	39.419
82.5852	123.2652	40.68	82.6068	123.4018	40.795	83.7794	123.1212	39.3419
82.5653	123.2745	40.7093	82.58	123.3854	40.8054	83.801	123.1083	39.3073
82.5679	123.2685	40.7006	82.6011	123.3912	40.7901	84.3067	123.0087	38.7019
82.6138	123.2338	40.6201	82.6657	123.368	40.7023	84.1959	123.0615	38.8656
82.7133	123.2026	40.4893	82.7125	123.3845	40.672	84.4184	123.0441	38.6257

84.4895	123.045	38.5556	83.0467	123.6114	40.5646	82.7809	123.8478	41.0669
84.4825	122.9567	38.4742	83.0054	123.6334	40.628	82.7584	123.8495	41.0911
84.8514	122.8944	38.0429	83.039	123.6798	40.6408	82.7341	123.8556	41.1215
84.5215	122.974	38.4525	82.9013	123.7655	40.8643	82.7737	123.8237	41.05
84.5302	122.9368	38.4066	83.0433	123.6755	40.6322	82.7748	123.8218	41.047
84.544	122.9229	38.3789	82.9853	123.7144	40.7292	82.7012	123.8365	41.1353
84.4695	123.0078	38.5383	83.097	123.6564	40.5594	82.6692	123.8608	41.1916
84.3911	123.0009	38.6099	83.0883	123.653	40.5646	82.6328	123.8928	41.26
84.1976	123.1169	38.9193	83.0545	123.6564	40.6019	82.6034	123.8729	41.2695
84.0244	123.142	39.1176	83.0124	123.659	40.6465	82.6103	123.892	41.2817
83.8945	123.1862	39.2917	82.89	123.704	40.814	82.6787	123.8798	41.2011
84.0643	123.1663	39.102	82.7471	123.7335	40.9864	82.6504	123.8457	41.1953
84.0695	123.1438	39.0743	82.5895	123.756	41.1665	82.6155	123.8764	41.2609
84.4392	123.0745	38.6353	82.5436	123.7707	41.2271	82.5938	123.8504	41.2565
84.7951	122.9697	38.1746	82.6423	123.7655	41.1232	82.5921	123.8902	41.2981
84.4836	123.0344	38.5509	82.6484	123.7508	41.1024	82.5627	123.8902	41.3276
83.9456	123.1827	39.2371	82.6778	123.756	41.0782	82.5514	123.9058	41.3544
83.9101	123.2018	39.2917	82.6169	123.7858	41.1689	82.5557	123.9179	41.3622
84.0132	123.1801	39.167	82.6614	123.7768	41.1154	82.5609	123.9708	41.4098
84.3266	123.116	38.7894	82.6449	123.8019	41.157	82.5447	123.9206	41.3759
84.195	123.1074	38.9124	82.567	123.8209	41.254	82.4787	123.9318	41.4531
83.9872	123.1793	39.1921	82.638	123.8253	41.1873	82.5055	123.9604	41.4549
84.2063	123.1195	38.9132	82.7289	123.8071	41.0782	82.5029	123.9318	41.4289
83.9196	123.1723	39.2527	82.6761	123.8209	41.1448	82.5566	123.9335	41.3769
83.8881	123.2097	39.3216	82.6943	123.7984	41.1041	82.5194	123.9015	41.3821
83.9344	123.2087	39.2743	82.735	123.8026	41.0676	82.5341	123.8868	41.3527
84.1266	123.155	39.0284	82.8043	123.795	40.9907	82.5098	123.9101	41.4003
84.1361	123.1767	39.0405	82.7783	123.7456	40.9673	82.5051	123.9365	41.4314
84.1656	123.1619	38.9964	82.7229	123.7785	41.0556	82.522	123.9153	41.3934
84.2366	123.1316	38.895	82.6908	123.769	41.0782	82.5272	123.9223	41.3951
84.0582	123.1905	39.1323	82.6475	123.7863	41.1388	82.5964	123.8703	41.2739
84.0547	123.1853	39.1306	82.7982	123.7499	40.9517	82.5843	123.8573	41.273
83.9814	123.2423	39.2609	82.8424	123.7837	40.9413	82.5912	123.8513	41.26
83.9924	123.2555	39.2631	82.7497	123.769	41.0193	82.5852	123.9205	41.3354
83.9655	123.2884	39.3228	82.7341	123.7893	41.0553	82.5272	123.9353	41.4081
83.9144	123.2858	39.3713	82.7151	123.7924	41.0773	82.5393	123.9231	41.3838
83.9681	123.2624	39.2943	82.6545	123.8106	41.1561	82.4945	123.9294	41.4349
83.8408	123.3499	39.509	82.6146	123.8565	41.2418	82.5055	123.9101	41.4046
83.7958	123.3247	39.5289	82.6597	123.8625	41.2029	82.5254	123.9145	41.389
83.7768	123.3438	39.567	82.7021	123.8461	41.144	82.4847	123.9361	41.4514
83.7727	123.3445	39.5718	82.6345	123.8452	41.2107	82.3514	123.9543	41.6029
83.3342	123.5014	40.1672	82.5921	123.8573	41.2652	82.3964	123.9249	41.5285
83.0017	123.6729	40.6712	82.5746	123.8307	41.2561	82.3618	123.9205	41.5588
82.9203	123.6538	40.7335	82.8865	123.7846	40.898	82.3462	123.9656	41.6194
83.1619	123.543	40.3811	82.696	123.8668	41.1708	82.2496	123.9734	41.7239
83.1576	123.5308	40.3733	82.6553	123.8963	41.241	82.2033	123.9777	41.7744
83.142	123.5577	40.4157	82.7298	123.8599	41.1301	82.3297	123.9119	41.5822

82.3661	123.9032	41.5371	82.4605	123.8279	41.3674	82.554	123.8279	41.2739
82.367	123.9257	41.5588	82.4458	123.8703	41.4245	82.4998	123.8484	41.3486
82.4094	123.9353	41.5259	82.4639	123.8764	41.4124	82.4215	123.8452	41.4237
82.4345	123.9153	41.4808	82.4579	123.8911	41.4332	82.3739	123.9136	41.5397
82.3903	123.8998	41.5094	82.4501	123.8807	41.4306	82.3185	123.9153	41.5969
82.3192	123.9417	41.6226	82.457	123.8859	41.4289	82.2622	123.9621	41.6999
82.3037	123.924	41.6203	82.4328	123.9162	41.4834	82.2968	123.9699	41.6731
82.3652	123.9179	41.5527	82.4152	123.918	41.5028	82.3185	123.9517	41.6332
82.4155	123.8521	41.4367	82.3618	123.9075	41.5458	82.3722	123.9699	41.5977
82.4605	123.8487	41.3882	82.4042	123.879	41.4748	82.3967	123.918	41.5213
82.4804	123.8443	41.3639	82.5713	123.8097	41.2384	82.3375	123.9335	41.596
82.5644	123.8339	41.2695	82.5627	123.8357	41.273	82.3955	123.95	41.5544
82.6207	123.8235	41.2029	82.5514	123.8149	41.2635	82.4276	123.9301	41.5025
82.6495	123.8325	41.183	82.5523	123.7958	41.2436	82.3462	123.9534	41.6073
82.5869	123.8175	41.2306	82.6406	123.7491	41.1085	82.3366	123.9413	41.6047
82.5549	123.8253	41.2704	82.6257	123.7338	41.1081	82.2977	123.9543	41.6566
82.5791	123.8547	41.2756	82.7099	123.717	41.0071	82.3297	123.9777	41.648
82.638	123.8729	41.2349	82.6475	123.7274	41.0799	82.3174	124.0008	41.6833
82.6579	123.8461	41.1881	82.6112	123.7543	41.1431	82.2604	124.0331	41.7727
82.6744	123.8539	41.1795	82.6345	123.7499	41.1154	82.2405	124.0461	41.8056
82.7315	123.801	41.0695	82.6172	123.7482	41.131	82.2336	124.0149	41.7813
82.7182	123.8351	41.1169	82.5757	123.7473	41.1717	82.4215	123.9846	41.5631
82.7142	123.7924	41.0782	82.5107	123.7759	41.2652	82.5237	123.9093	41.3856
82.7463	123.7699	41.0236	82.52	123.8017	41.2816	82.5211	123.9223	41.4012
82.7523	123.7846	41.0323	82.5003	123.8054	41.305	82.5272	123.937	41.4098
82.683	123.8218	41.1388	82.4847	123.8209	41.3362	82.5015	123.9241	41.4226
82.6475	123.8409	41.1933	82.4596	123.8504	41.3908	82.5488	123.937	41.3882
82.6146	123.8642	41.2496	82.47	123.8088	41.3388	82.6371	123.9058	41.2687
82.5514	123.9041	41.3527	82.4596	123.8002	41.3405	82.722	123.9275	41.2055
82.6011	123.8889	41.2878	82.5358	123.8513	41.3154	82.7307	123.9266	41.1959
82.6397	123.9093	41.2695	82.5462	123.892	41.3457	82.6735	123.8937	41.2202
82.5462	123.8764	41.3302	82.6786	123.8607	41.1821	82.6718	123.885	41.2132
82.5358	123.898	41.3622	82.7133	123.8279	41.1145	82.6744	123.8764	41.202
82.5912	123.8798	41.2886	82.7099	123.8279	41.118	82.6539	123.8924	41.2385
82.5757	123.8989	41.3232	82.6813	123.8348	41.1535	82.7437	123.8876	41.144
82.5731	123.8677	41.2947	82.6752	123.8184	41.1431	82.6778	123.8972	41.2193
82.5367	123.8132	41.2765	82.6545	123.7802	41.1258	82.6484	123.9119	41.2635
82.5523	123.7976	41.2453	82.6034	123.7802	41.1769	82.6207	123.898	41.2773
82.594	123.8105	41.2165	82.5272	123.8253	41.2981	82.5982	123.9171	41.3189
82.6016	123.8114	41.2098	82.47	123.8599	41.3899	82.6051	123.9318	41.3267
82.5765	123.8461	41.2695	82.4372	123.8959	41.4587	82.5687	123.9119	41.3431
82.6285	123.8244	41.1959	82.4527	123.8972	41.4445	82.528	123.8894	41.3613
82.5783	123.8478	41.2695	82.4674	123.866	41.3986	82.4918	123.9347	41.4429
82.5289	123.8547	41.3258	82.4137	123.9049	41.4912	82.4995	123.911	41.4116
82.528	123.8668	41.3388	82.3609	123.9396	41.5787	82.5064	123.9145	41.4081
82.4899	123.8547	41.3648	82.4475	123.8963	41.4488	82.4787	123.8972	41.4185
82.4813	123.8704	41.3891	82.5237	123.8729	41.3492	82.4544	123.9318	41.4774

82.4752	123.9699	41.4947	84.0244	123.1325	39.1081	83.4901	123.0286	39.5385
82.4813	123.9915	41.5103	83.9525	123.136	39.1834	83.6945	122.961	39.2666
82.4821	123.9829	41.5008	84.0236	123.1169	39.0934	83.6867	122.9541	39.2674
82.4962	123.9673	41.471	84.0755	123.0883	39.0128	83.7265	122.9732	39.2466
82.5003	123.9456	41.4453	84.0175	123.1585	39.141	83.5707	123.0346	39.464
82.4925	123.9569	41.4644	84.0871	123.1172	39.0301	83.5897	123.0234	39.4337
82.5609	123.9231	41.3622	84.0149	123.1377	39.1228	83.6157	123.0243	39.4086
82.4639	123.9422	41.4782	83.9915	123.1827	39.1912	83.6714	123.0415	39.3701
82.5064	123.9327	41.4263	83.8668	123.0286	39.1618	83.7776	122.9766	39.199
82.4847	123.8764	41.3916	83.7629	123.0554	39.2925	83.6685	123.0364	39.3679
82.5254	123.9006	41.3752	83.7629	123.0381	39.2752	83.6702	123.0139	39.3436
82.6116	123.8378	41.2262	83.6824	123.0381	39.3557	83.6538	123.0641	39.4103
82.5315	123.8738	41.3423	83.749	123.0087	39.2596	83.5897	123.0095	39.4198
82.4943	123.8781	41.3838	83.6793	123.0247	39.3454	83.2702	123.155	39.8849
82.4484	123.924	41.4756	83.7508	122.9879	39.2371	83.1377	123.1801	40.0425
82.3817	123.9136	41.5319	83.698	123.026	39.328	83.2441	123.2203	39.9762
82.3531	123.9361	41.583	83.704	123.0217	39.3176	83.2624	123.2269	39.9645
82.3427	123.9682	41.6255	83.5689	123.0693	39.5004	83.4945	123.116	39.6216
82.4518	123.9179	41.4661	83.5152	123.1135	39.5982	84.0149	122.9784	38.9635
82.4319	123.9039	41.4719	83.1905	123.2589	40.0684	83.9344	123.0494	39.115
82.4778	123.9119	41.4341	82.9939	123.3577	40.3637	83.7655	123.0546	39.2891
82.451	123.943	41.4921	83.2846	123.2176	39.933	83.5135	123.1221	39.6086
83.2113	123.6737	40.4625	83.2676	123.1992	39.9316	83.6893	123.0667	39.3774
83.6711	123.4209	39.7498	83.4165	123.1412	39.7246	83.6811	123.0608	39.3798
84.0348	123.2295	39.1947	83.2355	123.2104	39.9749	83.9058	123.0485	39.1427
83.8919	123.2728	39.3809	83.2095	123.2122	40.0026	84.0132	123.0061	38.9929
83.8478	123.2763	39.4285	83.2399	123.1983	39.9585	84.0365	123.032	38.9955
83.7815	123.3189	39.5375	83.0848	123.226	40.1412	84.2115	123.0069	38.7955
83.788	123.3118	39.5237	83.0554	123.2485	40.1931	84.1578	123.0191	38.8613
83.8841	123.2537	39.3696	83.112	123.2106	40.0986	84.3076	122.961	38.6534
83.7941	123.2771	39.483	83.1022	123.2364	40.1343	84.2314	123.0061	38.7747
83.7629	123.3048	39.5419	83.4901	123.0927	39.6025	84.2404	122.9956	38.7552
83.7768	123.2901	39.5134	83.4382	123.11	39.6718	84.0582	123.052	38.9938
83.8573	123.2321	39.3748	83.5109	123.0788	39.5679	84.0495	123.123	39.0734
83.7681	123.2927	39.5246	83.6001	123.045	39.4449	83.7361	123.2096	39.4735
83.689	123.3322	39.6432	83.4156	123.1057	39.69	83.8071	123.1827	39.3757
83.6425	123.3325	39.69	83.3351	123.1507	39.8156	83.7161	123.1949	39.4787
83.6902	123.2936	39.6034	83.6705	123.0106	39.3401	83.6702	123.2061	39.5359
83.8686	123.2494	39.3809	83.4503	123.0866	39.6363	83.7395	123.1663	39.4268
83.8763	123.2511	39.3748	83.3446	123.0797	39.735	84.5258	122.9023	38.3764
83.7846	123.2503	39.4657	83.4633	123.0433	39.58	84.3968	123.0043	38.6075
83.8971	123.207	39.3098	83.5629	122.9758	39.4129	84.2504	123.0416	38.7911
83.9422	123.149	39.2068	83.6373	122.9377	39.3003	84.0244	123.1169	39.0925
83.9361	123.1663	39.2302	83.5655	123.0052	39.4397	83.9049	123.1585	39.2536
83.8669	123.1577	39.2908	83.7802	122.9463	39.1661	84.0097	123.1065	39.0968
83.9881	123.1412	39.1531	83.4702	122.9974	39.5272	83.9093	123.155	39.2458
83.9647	123.1498	39.1851	83.3851	123.067	39.6819	83.8686	123.1481	39.2795

83.8652	123.1912	39.326	84.2721	122.9619	38.6898	82.7454	123.84	41.0946
83.8019	123.2252	39.4233	84.4522	122.9022	38.4499	82.7402	123.7958	41.0556
83.8556	123.2087	39.3531	84.4078	122.9313	38.5235	82.7298	123.8036	41.0738
84.0227	123.1983	39.1756	84.4626	122.9073	38.4447	82.6778	123.8209	41.1431
84.26	123.1316	38.8717	84.2193	123.0572	38.8379	82.6645	123.844	41.1795
83.8157	123.2373	39.4216	84.3474	123.0494	38.7019	82.7185	123.7785	41.06
83.6157	123.3022	39.6865	84.2582	123.058	38.7998	82.7012	123.7828	41.0816
83.6547	123.3092	39.6545	84.26	123.0277	38.7678	82.6882	123.801	41.1128
83.7923	123.2918	39.4995	84.2946	123.0398	38.7452	82.735	123.827	41.092
83.881	123.267	39.386	84.3197	123.0217	38.7019	82.6848	123.7872	41.1024
83.7984	123.2641	39.4657	84.3353	123.0407	38.7054	82.6787	123.808	41.1293
83.8495	123.2581	39.4086	84.3083	122.9956	38.6874	82.6597	123.8175	41.1578
83.6642	123.304	39.6398	84.3258	122.9983	38.6725	82.6285	123.827	41.1985
83.6503	123.3455	39.6952	84.2877	123.0381	38.7504	82.7147	123.7946	41.0799
83.691	123.3092	39.6181	84.3388	123.0234	38.6846	82.8432	123.7837	40.9405
83.7698	123.2633	39.4934	84.3665	122.9974	38.6309	82.8987	123.7491	40.8504
83.6694	123.3031	39.6337	84.4306	122.9602	38.5296	82.8822	123.7603	40.8781
83.6115	123.363	39.7515	84.4332	122.9325	38.4993	82.832	123.7898	40.9578
83.7854	123.1671	39.3817	84.4375	122.9316	38.4941	82.8554	123.8158	40.9604
83.7984	123.0684	39.27	84.4703	122.9093	38.439	82.903	123.7586	40.8556
83.9829	122.9688	38.986	84.3015	123.0043	38.7028	82.9307	123.7872	40.8565
84.1569	122.9203	38.7634	84.2504	123.0381	38.7877	82.8169	123.7541	40.9372
84.247	122.8848	38.6379	84.2297	123.0424	38.8128	82.7757	123.7915	41.0158
84.3076	122.8632	38.5556	84.318	123.0416	38.7236	82.748	123.814	41.066
84.1673	122.9342	38.7669	84.3907	122.9948	38.6041	82.7489	123.7984	41.0496
84.0281	122.9736	38.9455	84.4219	122.9654	38.5435	82.7116	123.7915	41.0799
84.0314	122.9749	38.9435	84.4323	122.9879	38.5556	82.7099	123.7612	41.0513
84.0374	122.968	38.9306	84.51	122.9613	38.4513	82.612	123.8149	41.2029
84.0019	122.9749	38.973	84.5189	122.9671	38.4482	82.6475	123.8469	41.1994
83.95	123	39.0501	84.486	122.9974	38.5114	82.683	123.8801	41.1971
83.9136	122.9784	39.0648	84.4583	123.0312	38.5729	82.7272	123.8703	41.1431
83.8668	123.0468	39.18	83.9326	123.2511	39.3185	82.8467	123.879	41.0323
83.9093	123.0684	39.1592	83.0978	123.5456	40.4477	82.7618	123.9023	41.1405
83.8775	123.0476	39.1701	82.9593	123.5326	40.5733	82.7047	123.9283	41.2236
83.9759	123.0069	39.031	82.8865	123.7205	40.834	82.6934	123.8746	41.1812
84.0045	123.0087	39.0042	82.8803	123.7567	40.8764	82.6345	123.827	41.1925
84.0842	122.9818	38.8976	82.7982	123.7716	40.9734	82.6623	123.801	41.1388
83.8902	123.0814	39.1912	82.7229	123.7231	41.0002	82.661	123.755	41.094
84.0132	123.0208	39.0076	82.7748	123.7603	40.9855	82.6778	123.7612	41.0834
84.0201	123.0312	39.0111	82.8467	123.7993	40.9526	82.677	123.7854	41.1085
84.0365	123.0502	39.0137	82.7644	123.8071	41.0427	82.5159	123.8062	41.2903
84.1277	122.9877	38.8601	82.6874	123.8313	41.144	82.4639	123.8244	41.3605
84.3509	122.9359	38.585	82.6319	123.827	41.1951	82.4406	123.8539	41.4133
84.163	122.9939	38.831	82.6293	123.8334	41.2041	82.4336	123.8582	41.4245
84.4375	122.8909	38.4534	82.6415	123.8322	41.1907	82.4709	123.8573	41.3864
84.3821	122.8961	38.514	82.6804	123.8417	41.1613	82.5429	123.8378	41.2949
84.4037	122.8814	38.4777	82.7047	123.8963	41.1916	82.5419	123.8833	41.3414

82.5791	123.9162	41.3371	82.5579	123.9215	41.3636	82.7437	124.4851	41.7415
82.5367	123.9379	41.4012	82.5358	123.9197	41.3838	82.7644	124.4315	41.667
82.5445	123.9075	41.3631	82.5185	123.911	41.3925	82.6936	124.4976	41.804
82.5176	123.8798	41.3622	82.5826	123.9309	41.3483	82.7696	124.3656	41.596
82.599	123.8625	41.2635	82.4562	123.9396	41.4834	82.8467	124.3362	41.4895
82.6475	123.8651	41.2176	82.4276	123.9335	41.5059	82.8813	124.3163	41.4349
82.6504	123.8739	41.2235	82.4769	123.9205	41.4436	82.8796	124.3544	41.4748
82.5973	123.9015	41.3042	82.4328	123.9231	41.4904	82.8891	124.3379	41.4488
82.5644	123.8807	41.3163	82.4628	123.9074	41.4446	82.8692	124.3492	41.48
82.5324	123.8989	41.3665	82.5341	123.937	41.4029	82.8363	124.3362	41.4999
82.6406	123.8781	41.2375	82.5324	123.8963	41.3639	82.8521	124.3804	41.5283
82.6986	123.8383	41.1396	82.528	123.898	41.37	82.9143	124.415	41.5008
82.7636	123.8513	41.0877	82.5358	123.9734	41.4375	82.9013	124.376	41.4748
82.7012	123.879	41.1777	82.5116	124.1266	41.6151	82.8406	124.389	41.5484
82.6848	123.8556	41.1708	82.8138	123.956	41.1422	82.8363	124.4133	41.577
82.625	123.8634	41.2384	83.2944	124.2288	40.9344	82.8562	124.4167	41.5605
82.5523	123.9396	41.3873	82.5438	124.3029	41.7591	82.9073	124.3812	41.4739
82.6406	123.8894	41.2488	82.4717	124.2816	41.8099	82.8943	124.4003	41.506
82.5246	123.8928	41.3683	82.4674	124.2938	41.8264	82.8821	124.413	41.5309
82.5202	123.892	41.3717	82.522	124.2418	41.7198	83.0511	124.3353	41.2843
82.541	123.9266	41.3856	82.528	124.2141	41.6861	83.0346	124.3111	41.2765
82.5419	123.9344	41.3925	82.4856	124.2505	41.7649	83.0433	124.3284	41.2851
82.5003	123.9223	41.4219	82.4743	124.2782	41.8038	83.0623	124.2202	41.1578
82.6143	123.8625	41.2482	82.4553	124.2773	41.822	83.0415	124.1942	41.1526
82.5376	123.8573	41.3198	82.4423	124.2998	41.8575	83.0779	124.1873	41.1093
82.47	123.8712	41.4012	82.3817	124.3126	41.9309	83.0814	124.1777	41.0963
82.5428	123.8296	41.2869	82.373	124.3068	41.9337	83.0277	124.1691	41.1414
82.5046	123.8972	41.3925	82.3072	124.3544	42.0472	83.0345	124.2104	41.1759
82.4769	123.9041	41.4271	82.3289	124.3553	42.0264	83.0069	124.2028	41.1959
82.4423	123.9292	41.4869	82.3263	124.344	42.0177	83.0242	124.2202	41.1959
82.4241	123.9231	41.499	82.3254	124.3708	42.0454	82.9983	124.2496	41.2514
82.38	123.8995	41.5195	82.2934	124.3682	42.0749	83.0138	124.2453	41.2314
82.3765	123.9335	41.557	82.3756	124.3466	41.971	83.0822	124.279	41.1968
82.4224	123.9353	41.5129	82.3579	124.317	41.9591	83.0225	124.3284	41.3059
82.5523	123.9067	41.3544	82.3635	124.3215	41.958	82.9688	124.3674	41.3986
82.5739	123.8937	41.3198	82.4224	124.3241	41.9017	82.9411	124.3575	41.4164
82.6484	123.8513	41.2029	82.5306	124.2868	41.7562	82.8761	124.428	41.5518
82.6025	123.8539	41.2514	82.5739	124.2903	41.7164	82.942	124.383	41.441
82.5549	123.8755	41.3206	82.6371	124.3042	41.667	82.9108	124.4046	41.4938
82.5879	123.8739	41.2861	82.7107	124.273	41.5622	83.097	124.3449	41.2479
82.5462	123.8634	41.3172	82.7004	124.273	41.5726	83.0467	124.3172	41.2704
82.4995	123.8946	41.3951	82.6988	124.3135	41.6146	83.0338	124.3197	41.286
82.502	123.8876	41.3856	82.6986	124.3232	41.6246	83.0355	124.3535	41.318
82.4882	123.911	41.4228	82.6623	124.3466	41.6843	83.0873	124.3417	41.2543
82.4527	123.9361	41.4834	82.6926	124.3232	41.6306	83.0883	124.305	41.2167
82.4232	123.9422	41.5189	82.6865	124.3449	41.6584	83.1307	124.3284	41.1977
82.5211	123.9517	41.4306	82.7636	124.3743	41.6107	83.1238	124.3275	41.2037

83.1714	124.3197	41.1483	83.1896	124.2037	41.0141	82.8777	124.1743	41.2966
83.2	124.3163	41.1163	83.1723	124.1743	41.002	82.8259	124.2228	41.3968
83.1333	124.3249	41.1916	83.1455	124.1864	41.0409	82.7792	124.2626	41.4834
83.1619	124.2842	41.1223	83.1264	124.1968	41.0704	82.7644	124.3362	41.5718
83.1613	124.2677	41.1063	83.1602	124.2002	41.0401	82.8112	124.3414	41.5302
83.1437	124.2894	41.1457	83.1013	124.2531	41.1518	82.8112	124.3146	41.5034
83.1697	124.2955	41.1258	83.1067	124.2263	41.1196	82.9324	124.2938	41.3613
83.1654	124.2383	41.073	83.0355	124.2479	41.2124	83.0381	124.2487	41.2107
83.1325	124.2427	41.1102	83.0147	124.2617	41.247	83.0838	124.2236	41.1398
83.1714	124.247	41.0756	83.058	124.2756	41.2176	83.2347	124.1466	40.9119
83.1688	124.2461	41.0773	83.0078	124.299	41.2912	83.1957	124.1561	40.9604
83.1992	124.2764	41.0773	83.0762	124.2912	41.215	83.1636	124.1466	40.9829
83.1745	124.2501	41.0755	83.1082	124.2513	41.1431	83.1671	124.1223	40.9552
83.2052	124.2548	41.0496	83.1585	124.2063	41.0479	83.2459	124.1691	40.9232
83.2485	124.2383	40.9898	83.1305	124.1857	41.0553	83.2511	124.1301	40.879
83.2338	124.2617	41.0279	83.0883	124.1795	41.0911	83.271	124.1171	40.8461
83.194	124.2401	41.0461	83.0667	124.1665	41.0998	83.2882	124.1126	40.8245
83.1533	124.2635	41.1102	83.0329	124.1864	41.1535	83.3741	124.0781	40.7041
83.1446	124.2591	41.1145	83.0303	124.1907	41.1604	83.4009	124.0755	40.6746
83.1385	124.2661	41.1275	83.0242	124.1873	41.163	83.3715	124.0764	40.7049
83.1622	124.2553	41.0931	83.0034	124.1682	41.1648	83.2901	124.0911	40.801
83.2199	124.208	40.9881	83.0848	124.1474	41.0626	83.3291	124.0652	40.7361
83.2719	124.2158	40.9439	83.1816	124.095	40.9134	83.3672	124.092	40.7248
83.2476	124.2236	40.976	83.226	124.0582	40.8322	83.3723	124.0712	40.6989
83.2754	124.2046	40.9292	83.2199	124.0652	40.8452	83.2838	124.1232	40.8394
83.2953	124.2228	40.9275	83.1992	124.0859	40.8868	83.4174	124.1171	40.6997
83.3161	124.2392	40.9232	83.1472	124.118	40.9708	83.4849	124.079	40.5941
83.2702	124.2505	40.9803	83.0667	124.1275	41.0608	83.5144	124.0721	40.5577
83.1895	124.2271	41.0376	83.0545	124.1431	41.0886	83.517	124.0894	40.5724
83.1992	124.2487	41.0496	82.9749	124.1639	41.189	83.4615	124.0816	40.6201
83.1152	124.2115	41.0963	83.0112	124.1604	41.1492	83.4875	124.0617	40.5742
83.0978	124.1976	41.0998	83.0177	124.1576	41.1398	83.5222	124.0461	40.5239
82.9783	124.2669	41.2886	83.0069	124.1691	41.1622	83.4599	124.0545	40.5945
82.9879	124.2739	41.286	83.0467	124.1543	41.1076	82.9091	122.0041	39.0951
83.0476	124.2669	41.2193	83.1585	124.0972	40.9387	82.9164	122.0302	39.1138
83.1567	124.2366	41.0799	83.1714	124.0331	40.8617	82.9428	122.0362	39.0934
83.1914	124.2297	41.0383	83.1645	124.0703	40.9058	82.929	122.0855	39.1566
83.2459	124.2069	40.961	83.174	124.053	40.879	82.9255	122.1479	39.2224
83.3005	124.1959	40.8954	83.1706	124.0548	40.8842	82.9593	122.1643	39.2051
83.3169	124.1777	40.8608	83.1648	124.0853	40.9205	83.0034	122.1453	39.1419
83.3109	124.1881	40.8773	83.1247	124.1145	40.9898	82.9705	122.1141	39.1436
83.3022	124.1821	40.8799	83.0736	124.1396	41.066	83.0234	122.2068	39.1834
83.2953	124.202	40.9067	83.0199	124.1457	41.1258	83.0043	122.2042	39.1999
83.2442	124.1795	40.9353	82.9255	124.2141	41.2886	83.0265	122.2971	39.2706
83.2139	124.1847	40.9708	82.8926	124.1639	41.2713	83.0156	122.309	39.2934
83.164	124.2078	41.0438	82.922	124.1976	41.2756	82.9671	122.3012	39.3341
83.1662	124.2046	41.0383	82.9151	124.1708	41.2557	82.9662	122.3531	39.3869

82.9411	122.3653	39.4242	82.8077	122.0821	39.2743	82.8142	122.2337	39.4194
82.9272	122.3176	39.3904	82.7514	122.1453	39.3938	82.7688	122.2172	39.4484
82.9143	122.4068	39.4926	82.7722	122.128	39.3557	82.7844	122.3124	39.5281
82.9065	122.4596	39.5532	82.8121	122.1358	39.3237	82.7965	122.2942	39.4978
82.8865	122.4962	39.6097	82.7956	122.2016	39.406	82.7532	122.425	39.6718
82.9515	122.4354	39.4839	82.7757	122.2068	39.4311	82.7982	122.3427	39.5445
82.942	122.4285	39.4865	82.7922	122.2715	39.4793	82.8207	122.2648	39.4441
82.9333	122.4501	39.5168	82.7644	122.1929	39.4285	82.806	122.2258	39.4198
82.9082	122.4874	39.5792	82.767	122.2925	39.5255	82.831	122.2557	39.4247
82.8969	122.4917	39.5948	82.7921	122.3038	39.5116	82.8207	122.3081	39.4874
82.9567	122.5107	39.5541	82.8181	122.3523	39.5341	82.8103	122.373	39.5627
82.9636	122.4865	39.5229	82.7965	122.3419	39.5454	82.7852	122.2951	39.5099
83.008	122.5015	39.4934	82.8424	122.3384	39.496	82.7618	122.309	39.5471
82.974	122.5298	39.5558	82.787	122.3046	39.5177	82.8207	122.2622	39.4415
82.9454	122.5012	39.5558	82.8142	122.2724	39.4582	82.7956	122.212	39.4164
82.961	122.4917	39.5307	82.845	122.0977	39.2527	82.7844	122.2345	39.4501
82.9428	122.5021	39.5592	82.8606	122.147	39.2865	82.7341	122.2742	39.5401
82.8796	122.5436	39.664	82.8536	122.1159	39.2622	82.7237	122.3038	39.58
82.8761	122.5003	39.6242	82.9316	122.1358	39.2042	82.7151	122.2951	39.58
82.8354	122.4475	39.6121	82.9307	122.0726	39.1419	82.7116	122.2258	39.5142
82.9076	122.4997	39.5921	82.8831	122.1618	39.2787	82.6969	122.2354	39.5385
82.9506	122.4588	39.5082	82.845	122.2042	39.3592	82.7376	122.2457	39.5082
83	122.4544	39.4545	82.8257	122.1544	39.3287	82.7757	122.3107	39.535
82.9619	122.4553	39.4934	82.7818	122.1288	39.3471	82.8034	122.2839	39.4804
82.9801	122.464	39.4839	82.8017	122.1392	39.3376	82.8283	122.1764	39.3481
82.9627	122.5168	39.5541	82.7999	122.2302	39.4302	82.7913	122.2198	39.4285
82.9705	122.4813	39.5108	82.8043	122.1436	39.3393	82.7558	122.1938	39.438
82.9558	122.4181	39.4623	82.8337	122.1947	39.3609	82.7489	122.1999	39.451
82.9261	122.4662	39.5401	82.8112	122.1643	39.3531	82.7887	122.1124	39.3237
82.9428	122.535	39.5922	82.8346	122.1791	39.3445	82.8329	122.154	39.3211
82.9766	122.4475	39.4709	82.8327	122.1729	39.3401	82.8528	122.0959	39.2432
82.9896	122.4874	39.4978	82.8138	122.225	39.4112	82.8363	122.147	39.3107
82.9688	122.5402	39.5714	82.864	122.186	39.322	82.809	122.083	39.2741
83.0164	122.5263	39.5099	82.8173	122.2553	39.438	82.7947	122.1427	39.348
82.9454	122.4441	39.4986	82.7887	122.2648	39.4761	82.8043	122.1306	39.3263
82.9653	122.5592	39.5939	82.8121	122.1782	39.3661	82.8008	122.0985	39.2977
82.9578	122.6142	39.6564	82.8398	122.2102	39.3705	82.8225	122.1098	39.2873
82.9168	122.4596	39.5428	82.8259	122.2293	39.4034	82.8121	122.0829	39.2709
82.9264	122.4778	39.5515	82.8609	122.2178	39.3569	82.7861	122.1167	39.3306
82.8831	122.3341	39.451	82.9091	122.1635	39.2544	82.7982	122.1115	39.3133
82.9394	122.412	39.4727	82.9091	122.1444	39.2354	82.7755	122.0822	39.3067
82.9281	122.2475	39.3194	82.8701	122.1228	39.2527	82.7298	122.0708	39.341
82.8813	122.1314	39.2501	82.8718	122.2206	39.3488	82.7159	122.1297	39.4138
82.8346	122.1756	39.341	82.9021	122.218	39.3159	82.6614	122.1202	39.4588
82.7956	122.1115	39.3159	82.8701	122.2518	39.3817	82.6943	122.1306	39.4363
82.7975	122.046	39.2485	82.8519	122.218	39.3661	82.6978	122.1695	39.4718
82.793	122.0223	39.2293	82.8251	122.3081	39.483	82.6839	122.1029	39.419

82.6449	122.1046	39.4597	82.7523	122.315	39.5627	82.8173	122.4233	39.606
82.6484	122.1185	39.4701	82.7185	122.2977	39.5792	82.8337	122.4683	39.6346
82.6125	122.1306	39.5181	82.7116	122.2865	39.5748	82.8562	122.4631	39.6069
82.6025	122.1185	39.516	82.7584	122.3202	39.5618	82.8614	122.4631	39.6017
82.6683	122.1436	39.4752	82.7878	122.2953	39.5075	82.8787	122.4709	39.5922
82.7237	122.1522	39.4285	82.8155	122.2908	39.4752	82.8493	122.4822	39.6329
82.6683	122.1505	39.4822	82.7748	122.3289	39.5541	82.838	122.49	39.652
82.6562	122.0821	39.4259	82.7965	122.3393	39.5428	82.8632	122.4553	39.5922
82.69	122.1773	39.4874	82.7748	122.373	39.5982	82.871	122.5064	39.6355
82.6813	122.0916	39.4103	82.7982	122.3471	39.5489	82.8484	122.5203	39.6718
82.6663	122.1949	39.5287	82.7618	122.4397	39.6779	82.8701	122.47	39.5999
82.6181	122.1124	39.4943	82.774	122.4103	39.6363	82.8684	122.4951	39.6268
82.6094	122.2224	39.6129	82.7843	122.4063	39.622	82.8987	122.412	39.5134
82.6293	122.1834	39.5541	82.8034	122.4293	39.6259	82.8987	122.3627	39.464
82.6285	122.1877	39.5592	82.7445	122.3834	39.6389	82.8653	122.4178	39.5524
82.6614	122.1375	39.4761	82.7523	122.4544	39.7021	82.8051	122.4553	39.6502
82.6371	122.1626	39.5255	82.7636	122.4484	39.6848	82.8476	122.4345	39.587
82.6484	122.1618	39.5134	82.7445	122.4389	39.6943	82.8458	122.4458	39.5999
82.6381	122.1747	39.5366	82.7826	122.4207	39.6381	82.871	122.4311	39.5601
82.6501	122.199	39.5489	82.7601	122.4345	39.6744	82.8268	122.4778	39.651
82.6415	122.1773	39.5359	82.7385	122.4794	39.741	82.8545	122.4596	39.6051
82.5835	122.2535	39.6701	82.7445	122.4042	39.6597	82.8225	122.4354	39.6129
82.5523	122.2085	39.6562	82.7246	122.4034	39.6788	82.8636	122.4072	39.5436
82.5817	122.1721	39.5904	82.7592	122.4726	39.7134	82.8103	122.4215	39.6112
82.5705	122.2224	39.6519	82.7748	122.3696	39.5948	82.8502	122.5627	39.7125
82.5549	122.1999	39.645	82.7558	122.4354	39.6796	82.8251	122.4943	39.6692
82.6284	122.3033	39.6749	82.8086	122.4172	39.6086	82.8095	122.4276	39.6181
82.6259	122.2726	39.6467	82.8406	122.4709	39.6303	82.8173	122.4648	39.6476
82.6995	122.2163	39.5168	82.8521	122.409	39.5568	82.8675	122.4311	39.5636
82.7315	122.2042	39.4727	82.8597	122.4441	39.5844	82.8441	122.4189	39.5748
82.7203	122.2094	39.4891	82.8935	122.3912	39.4978	82.8424	122.4539	39.6115
82.7246	122.2709	39.5463	82.9047	122.4103	39.5056	82.8519	122.4363	39.5844
82.7454	122.2916	39.5463	82.8787	122.393	39.5142	82.8822	122.3471	39.4649
82.7965	122.2518	39.4553	82.9073	122.4094	39.5021	82.8857	122.3765	39.4908
82.7913	122.2654	39.474	82.9134	122.3575	39.4441	82.877	122.4267	39.5497
82.7558	122.27	39.5142	82.89	122.4544	39.5644	82.9255	122.4337	39.5082
82.7627	122.2925	39.5298	82.8645	122.4557	39.5912	82.948	122.3713	39.4233
82.7237	122.3124	39.5887	82.8926	122.4622	39.5696	82.9584	122.3609	39.4025
82.7272	122.2414	39.5142	82.838	122.4735	39.6355	82.9349	122.4354	39.5005
82.7315	122.2761	39.5445	82.8008	122.5012	39.7004	82.9307	122.3661	39.4354
82.7168	122.2267	39.5099	82.8562	122.4423	39.5861	82.9134	122.3904	39.477
82.7099	122.3462	39.6363	82.877	122.5073	39.6303	82.9125	122.3713	39.4588
82.7218	122.2953	39.5736	82.832	122.5549	39.7229	82.8701	122.3488	39.4787
82.7133	122.2683	39.5549	82.8311	122.4709	39.6398	82.89	122.3895	39.4995
82.7125	122.2908	39.5783	82.8134	122.4883	39.6749	82.8883	122.4086	39.5203
82.7471	122.2916	39.5445	82.7965	122.412	39.6155	82.8813	122.4467	39.5653
82.7627	122.302	39.5393	82.8129	122.47	39.6571	82.8653	122.4266	39.5612

82.8225	122.4241	39.6017	82.774	122.4865	39.7125	82.6848	122.3479	39.6632
82.7852	122.4345	39.6493	82.8803	122.4618	39.5815	82.6978	122.3375	39.6398
82.7636	122.4761	39.7125	82.7289	122.3107	39.5818	82.7731	122.3453	39.5722
82.7991	122.4302	39.6311	82.793	122.3098	39.5168	82.8098	122.3429	39.5331
82.8216	122.406	39.5844	82.8484	122.2743	39.4259	82.8138	122.3462	39.5324
82.8684	122.3895	39.5211	82.8502	122.289	39.4389	82.8441	122.3211	39.477
82.8796	122.4068	39.5272	82.8354	122.2778	39.4423	82.7965	122.3064	39.5099
82.9229	122.4008	39.4778	82.832	122.2951	39.4631	82.8684	122.2908	39.4224
82.9517	122.3658	39.4141	82.8233	122.2934	39.4701	82.8424	122.3159	39.4735
82.9896	122.3644	39.3748	82.793	122.2916	39.4986	82.8545	122.3237	39.4692
82.9705	122.3644	39.3938	82.8081	122.3182	39.5102	82.8484	122.3427	39.4943
82.9472	122.3609	39.4138	82.806	122.2683	39.4623	82.871	122.3168	39.4458
82.9757	122.3107	39.335	82.8207	122.2864	39.4657	82.8195	122.3244	39.5049
83.0684	122.3315	39.2631	82.7913	122.283	39.4917	82.8242	122.354	39.5298
83.058	122.3098	39.2518	82.7549	122.3072	39.5523	82.8242	122.3401	39.516
83.0545	122.3159	39.2614	82.7575	122.3046	39.5471	82.8329	122.3367	39.5038
83.0618	122.3332	39.2714	82.8259	122.2986	39.4727	82.8536	122.3601	39.5064
83.0459	122.3644	39.3185	82.7965	122.2977	39.5012	82.8813	122.3297	39.4484
83.0424	122.3479	39.3055	82.7878	122.3226	39.5348	82.8909	122.3124	39.4216
83.0978	122.3583	39.2605	82.793	122.315	39.522	82.9117	122.3323	39.4207
83.0736	122.3618	39.2882	82.7514	122.322	39.5705			
83.0502	122.3349	39.2847	82.7445	122.3228	39.5783			
83.1074	122.3116	39.2042	82.7064	122.3064	39.5999			
83.0727	122.3306	39.2579	82.7159	122.3471	39.6311			
83.053	122.3024	39.2494	82.7809	122.3419	39.561			
83.0996	122.322	39.2224	82.7575	122.3254	39.5679			
83.0381	122.3211	39.283	82.7658	122.357	39.5912			
83.0632	122.3185	39.2553	82.7411	122.3393	39.5982			
83.084	122.3323	39.2484	82.7575	122.3419	39.5844			
83.0545	122.386	39.3315	82.7714	122.3583	39.587			
83.0649	122.3488	39.2839	82.7575	122.3323	39.5748			
82.9601	122.3808	39.4207	82.722	122.3575	39.6355			
82.9596	122.3852	39.4256	82.6943	122.3696	39.6753			
82.9498	122.3687	39.419	82.6752	122.3514	39.6762			
82.9298	122.4302	39.5004	82.698	122.3614	39.6634			
82.9428	122.4189	39.4761	82.6675	122.3514	39.6839			
82.9342	122.4276	39.4934	82.7021	122.38	39.6779			
82.8917	122.4008	39.509	82.69	122.373	39.6831			
82.858	122.3912	39.5333	82.6848	122.3817	39.6969			
82.8441	122.38	39.5359	82.7463	122.3523	39.606			
82.8204	122.4248	39.6044	82.7558	122.3332	39.5774			
82.7913	122.4337	39.6424	82.735	122.3436	39.6086			
82.754	122.4293	39.6753	82.7367	122.3174	39.5806			
82.7237	122.4562	39.7324	82.722	122.3315	39.6095			
82.7601	122.4527	39.6926	82.7125	122.3202	39.6077			
82.7385	122.47	39.7316	82.6952	122.3315	39.6363			
82.767	122.4891	39.7221	82.6926	122.3185	39.6259			

Table A.5 : weighted syringe		45	181.0812	90	165.3492	135	151.087
Time(min)	mmHg	46	180.3838	91	165.036	136	150.7423
1	218.95	47	179.9273	92	164.7752	137	150.6299
2	214.68	48	179.6504	93	164.5115	138	150.1093
3	212.04	49	179.259	94	164.2071	139	150.0012
5	209.83	50	179.0298	95	164.0137	140	149.6571
6	208.3	51	178.484	96	163.6458	141	149.1915
7	206.86	52	177.9055	97	163.2462	142	149.0317
8	205.65	53	177.6589	98	162.8455	143	148.7439
9	204.46	54	177.233	99	162.5805	144	148.3753
10	203.21	55	176.811	100	162.0779	145	148.0959
11	202.3	56	176.3679	101	161.8876	146	147.5288
12	201.27	57	175.811	102	161.8684	147	147.2822
13	200.35	58	175.6312	103	161.49	148	147.1424
14	199.73	59	175.302	104	161.2459	149	146.7285
15	198.69	60	174.8506	105	160.5396	150	146.6057
16	197.78	61	174.3716	106	160.3155	151	146.3033
17	196.99	62	173.99	107	159.8565	152	146.0971
18	196	63	173.4758	108	159.6382	153	145.8319
19	195.49	64	173.3824	109	159.3272	154	145.4129
20	194.82	65	172.784	110	158.9871	155	145.1309
21	194.08	66	172.4068	111	158.9047	156	144.7524
22	193.19	67	172.1561	112	158.4544	157	144.5713
23	192.54	68	171.5845	113	158.1571	158	144.2425
24	192	69	171.3841	114	157.7984	159	144.0555
25	191.34	70	170.8575	115	157.5629	160	143.6622
26	190.77	71	170.6497	116	157.2732	161	143.4298
27	190.18	72	170.3527	117	156.8806	162	143.2827
28	189.62	73	170.2541	118	156.4867	163	143.0633
29	189.15	74	169.8262	119	156.0214	164	142.7087
30	188.45	75	169.5719	120	155.8841	165	142.5227
31	187.94	76	169.4818	121	155.6152	166	142.1136
32	187.32	77	169.0145	122	155.4636	167	141.9763
33	186.66	78	168.7429	123	155.1561	168	141.4958
34	186.25	79	168.5544	124	154.8401	169	141.341
35	185.55	80	168.2443	125	154.4671	170	141.0671
36	185.03	81	167.9755	126	154.0703	171	140.7205
37	184.43	82	167.6244	127	153.9207	172	140.4361
38	183.99	83	167.3106	128	153.4801	173	140.319
39	183.48	84	166.9933	129	153.1682	174	140.0205
40	182.88	85	166.3691	130	152.7995	175	139.7181
41	182.53	86	166.5751	131	152.4962	176	139.4866
42	182	87	166.0463	132	152.0202	177	139.1166
43	181.58	88	165.5293	133	151.8173	178	138.9547
44	181.2	89	165.4641	134	151.5451	179	138.5903

180	138.34	225	128.5714	270	120.278	315	113.5115
181	138.23	226	127.9874	271	120.1263	316	113.3827
182	138.11	227	127.6422	272	119.9236	317	113.3072
183	137.84	228	127.7147	273	119.7374	318	113.2598
184	137.56	229	127.6121	274	119.5527	319	113.0321
185	137.31	230	127.5189	275	119.3846	320	112.9743
186	136.93	231	127.2948	276	119.2982	321	112.8605
187	136.76	232	127.1388	277	119.0955	322	112.7438
188	136.53	233	127.0079	278	118.9441	323	112.6541
189	136.42	234	126.7966	279	118.801	324	112.4494
190	136.03	235	126.5642	280	118.7362	325	112.2606
191	135.91	236	126.398	281	118.4883	326	112.1861
192	135.64	237	126.2672	282	118.31	327	111.9834
193	135.32	238	126.1539	283	118.2208	328	111.8708
194	135.05	239	125.9021	284	118.048	329	111.7332
195	134.9	240	125.6857	285	117.8734	330	111.6338
196	134.69	241	125.4903	286	117.7677	331	111.4912
197	134.47	242	125.2833	287	117.6374	332	111.3663
198	134.16	243	125.1227	288	117.4454	333	111.2777
199	134.05	244	125.051	289	117.372	334	111.1208
200	133.76	245	124.7147	290	117.0854	335	110.9357
201	133.56	246	124.5651	291	116.9589	336	110.9205
202	133.3	247	124.4846	292	116.8226	337	110.7264
203	133.15	248	124.2522	293	116.7216		
204	132.9	249	124.0663	294	116.4858		
205	132.65	250	123.8364	295	116.3649		
206	132.39	251	123.6216	296	116.1799		
207	132.31	252	123.5399	297	116.1001		
208	132.03	253	123.3954	298	116.0087		
209	131.9	254	123.0747	299	115.8289		
210	131.69	255	122.8197	300	115.6243		
211	131.44	256	122.8122	301	115.5045		
212	131.19	257	122.6105	302	115.3534		
213	130.98	258	122.4087	303	115.2734		
214	130.87	259	122.2153	304	115.0175		
215	130.62	260	122.0698	305	114.8814		
216	130.49	261	121.9446	306	114.7801		
217	130.17	262	121.6759	307	114.5727		
218	130.05	263	121.5427	308	114.4062		
219	129.75	264	121.3939	309	114.3247		
220	129.56	265	121.1901	310	114.1373		
221	129.23	266	120.9779	311	114.0071		
222	129.06	267	120.7944	312	113.9003		
223	128.95	268	120.6003	313	113.7152		
224	128.51	269	120.4542	314	113.5735		

APPENDIX B: Qualitative Leak Diagnostic Testing

Introduction

The pressure test of the bioreactor chamber indicated the presence of a small leak within the system. The location of the leak has yet to be determined. In an attempt to isolate the source, smaller and simpler systems will be assembled, monitored for drops in pressure, and inspected for leaks. The tests will be conducted under semi-static conditions. Meaning, the systems will not have fluid flow and will undergo an initial pressurization. This simplification is justified in terms of reducing variables for the source of the leakage. If the system cannot maintain constant pressures in a static state, then it will not be able to with pulsatile flow.

Materials and Methods Setup 1

The first setup will include a 12” length of silicone tubing, 2 barbed male luer lock fittings, a pressure transducer and related data acquisition software, 2 female luer lock plugs, 1 two-way stop valve, and a luer lock style syringe. The two barbed luer lock fittings are each inserted into the 12” length of silicone tubing. On one end the pressure transducer was attached, and the two-way valve was attached to the opposing end of the tubing. Once the transducer is attached to the open system, it is zeroed using the bridge pod. The syringe is filled with water and connected to the stop valve. If the stop valve is closed, it is opened to allow water to flow through the length of tubing. Once water shoots out from the transducer side of the system, the transducer is capped with a female luer lock plug. Before removing the syringe, the stop valve is closed to prevent water from exiting the system.

The syringe is filled with water once again and attached to the stop valve. Water is then forced into the system once the valve is opened. The amount of water introduced is determined by the pressure reading on the data acquisition software. Once the pressure reaches 120 mmHg, the stop valve is closed to maintain a constant pressure. The pressure will be monitored until it drops 10 mmHg. The 10 mmHg limit is based on the presumption that if the system was to drop 10 mmHg within 24 hours it would not be an effective system capable of maintaining an acceptably constant pressure.

Results and Discussion

The pressure dropped at roughly 1 mmHg every two minutes. Since the pressure declined without the bioreactor chamber in place, it is safe to conclude that the system is subject to significant pressure loss independent of the bioreactor chamber. In fact the chamber might not be the source of the leak. It seems incongruous that the leak should be more pronounced without the bioreactor chamber. This could be attributed to simple connection variability either between the silicone tubing and the barbs or the luer lock fittings themselves. Accordingly, modifications would be made to Setup 1 to eliminate one of the variables.

Methods and Materials Setup 2

Setup 2 includes all of the parts described in Setup 1 except the addition of spring clamps. The spring clamps are brass loops used to secure micro-tubing over barbed fitting and prevent slippage. The hope here is to have the clamps squeeze the tubing and prevent the possibility of fluid pushing around the barb over time. Previous leak testing trials indicated a tendency for the silicone to balloon around the barb and then expel fluids.

In order to apply the spring clamps, the two barbed male luer lock fittings must be removed from the 12” length of silicone tubing. Using vice clamps, pinch the “ears” of the spring clamp and slide it onto the tubing. Repeat this process for both spring clamps. Once this is completed, re-attach the barbed fittings that had been previously removed. Use the vice clamp again to pinch the ears of the spring clamp and slide it over the barb. It should squeeze the tubing just behind the edge of the barb. Repeat the process for both barbs. Once this portion of the setup is complete, zero the pressure transducer using the bridge pod. It is ok if the transducer is not exactly zero, it is rather sensitive.

Take the luer lock syringe full of water and inject it into the system as described before. Be sure to cap off the end of the transducer and to turn off the two-way valve. Refill the syringe with fluid. Start recording live data with the data acquisition software and after attaching the syringe to the two-way valve inject the fluid into the system. Inject enough fluid to raise the pressure up to 120 mmHg. Accurately injecting enough fluid is possible using the display on the software. Once the appropriate pressure is reached, close the two-way valve. The pressure will be monitored once again until 10 mmHg of pressure is lost from the system.

Results and Discussion

The pressure dropped at roughly 1 mmHg per 3 minutes. The rate of decrease was not substantially different from the rate recorded for setup 1. Upon disassembly, it was found that the spring clamps were slightly deformed. The deformations were likely due to using the vice clamps to apply the spring clamps. Since they are deformed, their effectiveness for sealing the tube to barb connection was compromised. Given the slight improvement that was realized, it would be worthwhile to pursue enhancing the integrity of the seal between the tubing and barb connection.

Methods and Materials Setup 3

Setup 3 is the same as setup 1 except with the addition of twist ties. String or twine was a potential option. Using masonry string, it was found that the quality of the string made it slick and difficult to tie a tight knot that would not slip and loosen on the tube over the barb. Twist ties do not fall victim to slippage. Toying around with grocery store style paper covered twist ties, proof of concept was achieved. A tight seal can be achieved using the twist ties. The only issue is that the paper on these twist ties rips and tears. It’s not too bad, but eventually the wire gets worn out and breaks or doesn’t twist so well without the paper. As a remedy, heavy duty twist ties were used for securing electrical cords.

The parts are put together the same as they were for the first setup. This time the barbed fittings do not have to be removed like they were to accommodate the spring clamps. Once everything is in place, the pressure transducer is zeroed. The twist ties are then placed at each end of the silicone tubing. The tie is tightened just behind the edge of the barb. The tie is twisted until its ability to squeeze the tubing has been maxed out.

The luer lock syringe is filled with fluid, and the fluid is injected into the system. Once water shoots out the end of the transducer, the transducer is capped and the two-way valve is closed. Based on the pressure readings displayed on the data acquisition software, inject additional fluid into the closed system until 120 mmHg is shown. Then close off the two-way valve for a final time and monitor the system until it drops 10 mmHg.

Results and Discussion

The system lost 1 mmHg per every 10 minutes. The rate of pressure loss from the system has dramatically improved with the enhanced seal. Accordingly, it appears that some pressure continues to be lost through the connection between the tubing and barbed fittings. Nonetheless, there is still an unacceptable loss in pressure. Further tightening of the twist ties is impractical and unlikely to yield much more improvement in the rate of leakage. The next option is exploring the luer lock connections. They are the last remaining variable. The rate of leakage has been somewhat erratic over the test series and the rate here is half that of the original setup with the bioreactor chamber. This suggests that either the bioreactor chamber is contributing to the leakage or the additional connections to the chamber are leaking. Given the empirical evidence from previous trials conducted, the leaks are independent of the new bioreactor chamber. The next setup to be tested will become even simpler in order to isolate the luer lock fitting variable.

Methods and Materials Setup 4

This is the final setup to test whether or not the luer lock connections leak. There are only a few parts required for the test since it has been reduced greatly in complexity. The simplification is done in order to help reduce the number of variables affecting the drop in pressure. It is appropriate to note that the vendor supplying the luer lock fittings claims an air-tight connection to 20 psi.

This setup requires a single ADI pressure transducer, two stop valves, and one luer lock style syringe. The ADI pressure transducer is calibrated as usual. Using the bridge pod, zero the transducer. Once this is complete, attach both of the stop valves to each end of the ADI instrument. Make sure they are in the open position. Attaching the valves while they are closed will pressurize the interior of the system. Check that the transducer still reads zero once the valves have been added. Fill the syringe full of water and screw it on to one of the stop valves. Inject fluid through the system. Once the water shoots out the opposite stop valve, close both valves (starting with the farthest valve from the syringe). Refilling the syringe will not be necessary since the volume of the system is much smaller than that of the syringe. Press the "start" button on the LabChart software so it begins collecting data. Once the software begins collecting data, open the valve the syringe is attached to and inject water into the system until the desired 100 mmHg pressure is achieved. This will be a small amount. Hold it steady by applying pressure to the syringe plunger while closing the valve.

Since this is a qualitative test, watch the pressure drop until it loses 10 mmHg. At this point, the system will be outside the acceptable range for fluctuation.

Results and Discussion

The system leaks quickly – roughly 1 mmHg per second. The results indicate that the luer lock fittings are at least causing some of the issues with the dropping pressure measurements. Some future testing should be conducted to see if different fittings leak at different rates. There are a variety of valves and fittings used in the bioreactor system and potentially substitutes. For example, there are multiple 3 way valves to pick from. The potential source of leakage could be the ADI pressure transducer itself. Multiple ADI transducers should be tested as well to test whether or not the pressure drop depends on the transducer being used.

It is problematic that the luer lock fittings and/or pressure transducers are leaking. The ADI transducer is the only means of taking live pressure measurements within the system. If the leak can be accurately measured from the smallest system tested, the system can be rebuilt and compared to the initial leak. If there is a discrepancy at any stage where the larger system leaks more than the smaller system did, then additional leaks exist beyond the luer lock fittings. In which case, the system can be slowly rebuilt with the intention of locating and addressing the potential additional leaks.

Logically, the pressures drop faster with the smaller systems because with larger volumes, a micro leak at a specific rate represents a smaller percentage of the overall volume than is the case with the same micro leak and a much smaller volume.

Methods and Materials Setup 5

This test seeks to determine whether or not different luer lock valves leak at different rates. To perform the test, three different luer lock valves were used: two different three-way valves, and one two-way valve (Figure A.1). The ADI pressure transducer was filled with water and then capped at one end using a luer lock plug. Next, the valve in question would be filled with water and attached to the pressure transducer. Depending on the style of the valve, a luer lock plug would be added to the extra port of the three-way valve in order to provide a seal during the test. The plug would be added while the valve was open in order to prevent any excess pressure buildup beyond that applied intentionally by the syringe. The two-way valve would not require any modifications. Once the data acquisition software was enabled, a syringe full of water would be attached to the valve. Fluid would then be injected into the confined space to elevate the internal pressure. Once the pressure was elevated to 100 mmHg, the valve would be closed. Pressure would be monitored on the LabChart software. The pressure would be allowed to drop 10 mmHg, and the length of time that this would require was recorded. The average time for each valve would then be compared to the others, and the shortest duration would indicate the leakiest valve. The fact that the pressure is dropping would also indicate an issue with the luer lock interface and seal.

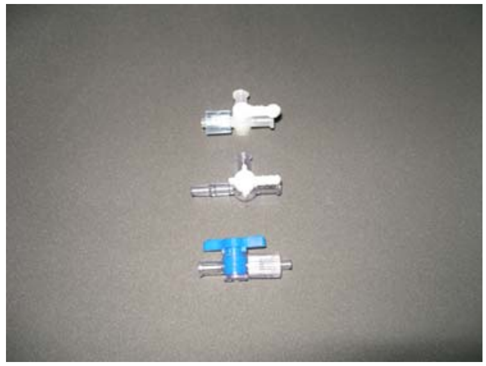

Figure A.1 The three different valves used to assess potential pressure.

Results and Discussion:

Out of the three valves tested, the two-way valve had the slowest rate of leakage. The three-way valves both leaked quite fast. The two-way valve leak was on the order of minutes, while the others were on the order of seconds. This is a somewhat surprising find because luer lock valves and fittings are marketed and represented as being pressure and leak tight up to 20 psi. 20 psi would be roughly equivalent to 1,000 mmHg, and well above the intended operational pressures of the bioreactor. Perhaps there is a minimum pressure required for the fittings to seal properly. Regardless, the fittings are an integral component of the current bioreactor system. Replacing them would not make sense – they are top of the line products used for this very purpose. There might be a way to ensure an adequate seal using Teflon tape and o-rings, but a quantitative look at the system is required to assess the practicality of repairing leaks.

Table A.6 General liner model ANOVA table.

General Linear Model: Pressure Drop versus Size, Treatment, Transducer

Factor	Type	Levels	Values
Size	fixed	2	12, 24
Treatment	fixed	3	a, b, c
Transducer	random	6	1, 2, 3, 4, 5, 6

Analysis of Variance for Pressure Drop, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Size	1	2.4200	2.4200	2.4200	276.50	0.000
Treatment	2	21.8536	21.8536	10.9268	1248.45	0.000
Size*Treatment	2	0.5625	0.5625	0.2812	32.13	0.000
Transducer	5	0.0428	0.0428	0.0086	0.98	0.439
Error	61	0.5339	0.5339	0.0088		
Total	71	25.4128				

S = 0.0935536   R-Sq = 97.90%   R-Sq(adj) = 97.55%

Table A.7 Least squares means for pressure drop.

Least Squares Means for Pressure Drop

Treatment		Mean
a		0.6708
b		0.2500
c		1.5708
Size		
12		1.0139
24		0.6472
Size*Treatment		
12	a	0.9167
12	b	0.3083
12	c	1.8167
24	a	0.4250
24	b	0.1917
24	c	1.3250

Table A.8 Tukey pairwise comparison of treatment factors.

```
Tukey Simultaneous Tests
Response Variable Pressure Drop
All Pairwise Comparisons among Levels of Size*Treatment
Size = 12
Treatment = a  subtracted from:

                 Difference       SE of            Adjusted
Size  Treatment    of Means  Difference  T-Value    P-Value
12    b             -0.6083     0.03819   -15.93     0.0000
12    c              0.9000     0.03819    23.56     0.0000
24    a             -0.4917     0.03819   -12.87     0.0000
24    b             -0.7250     0.03819   -18.98     0.0000
24    c              0.4083     0.03819    10.69     0.0000


Size = 12
Treatment = b  subtracted from:

                 Difference       SE of            Adjusted
Size  Treatment    of Means  Difference  T-Value    P-Value
12    c              1.5083     0.03819   39.492     0.0000
24    a              0.1167     0.03819    3.055     0.0374
24    b             -0.1167     0.03819   -3.055     0.0374
24    c              1.0167     0.03819   26.619     0.0000


Size = 12
Treatment = c  subtracted from:

                 Difference       SE of            Adjusted
Size  Treatment    of Means  Difference  T-Value    P-Value
24    a              -1.392     0.03819   -36.44     0.0000
24    b              -1.625     0.03819   -42.55     0.0000
24    c              -0.492     0.03819   -12.87     0.0000


Size = 24
Treatment = a  subtracted from:

                 Difference       SE of            Adjusted
Size  Treatment    of Means  Difference  T-Value    P-Value
24    b             -0.2333     0.03819   -6.109     0.0000
24    c              0.9000     0.03819   23.564     0.0000


Size = 24
Treatment = b  subtracted from:

                 Difference       SE of            Adjusted
Size  Treatment    of Means  Difference  T-Value    P-Value
24    c               1.133     0.03819    29.67     0.0000
```

Table A.9 Data measurements for pressure leakage.

Transducer	Size	Treatment	Pressure Drop (mmHg)	Transducer	Size	Treatment	Pressure Drop (mmHg)
1	12	A	1	1	24	a	0.5
2	12	A	0.8	2	24	a	0.4
3	12	a	0.9	3	24	a	0.5
4	12	a	0.8	4	24	a	0.4
5	12	a	0.9	5	24	a	0.4
6	12	a	0.9	6	24	a	0.5
1	12	b	0.2	1	24	b	0.2
2	12	b	0.2	2	24	b	0.2
3	12	b	0.3	3	24	b	0.2
4	12	b	0.3	4	24	b	0.3
5	12	b	0.3	5	24	b	0.3
6	12	b	0.3	6	24	b	0.2
1	12	a	0.9	1	12	c	1.8
2	12	a	0.9	2	12	c	1.8
3	12	a	1	3	12	c	1.7
4	12	a	1.2	4	12	c	1.6
5	12	a	0.8	5	12	c	1.9
6	12	a	0.9	6	12	c	2
1	12	b	0.2	1	24	c	1.4
2	12	b	0.4	2	24	c	1.1
3	12	b	0.4	3	24	c	1.4
4	12	b	0.4	4	24	c	1.3
5	12	b	0.4	5	24	c	1.5
6	12	b	0.3	6	24	c	1.3
1	24	a	0.4	1	12	c	1.8
2	24	a	0.4	2	12	c	1.9
3	24	a	0.4	3	12	c	1.9
4	24	a	0.4	4	12	c	1.8
5	24	a	0.3	5	12	c	1.8
6	24	a	0.5	6	12	c	1.8
1	24	b	0.2	1	24	c	1.3
2	24	b	0.2	2	24	c	1.1
3	24	b	0.1	3	24	c	1.4
4	24	b	0.1	4	24	c	1.3
5	24	b	0.1	5	24	c	1.4
6	24	b	0.2	6	24	c	1.4

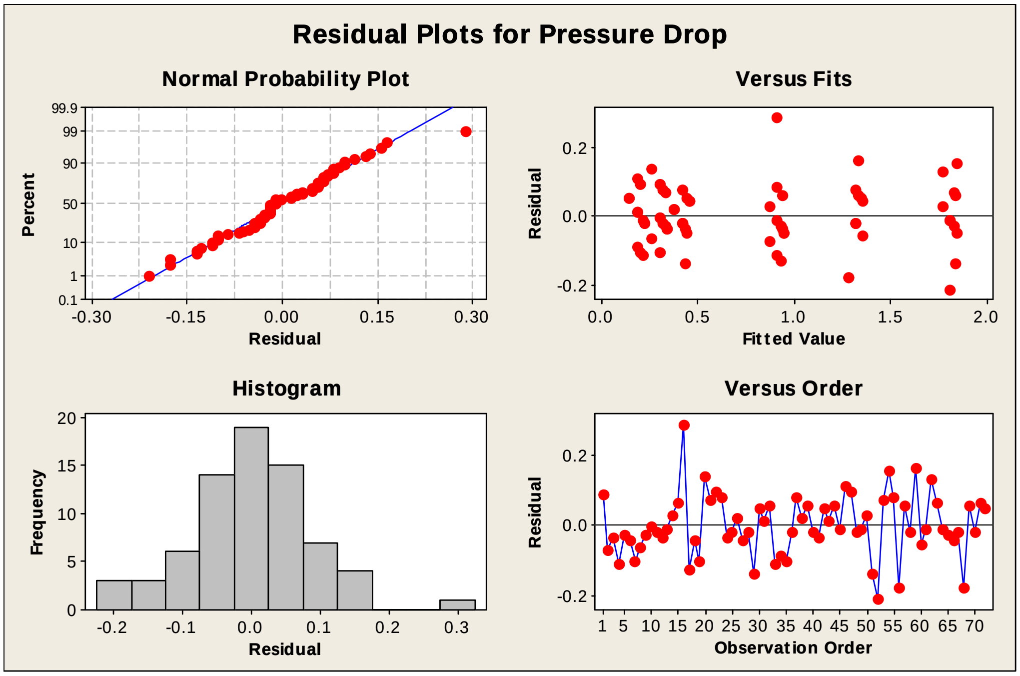

Figure A.2 Statistical charts used for assessing quality of data.

APPENDIX D: Design Considerations

Considerations for Future Prototype

Before completely committing to a design, it is important to first test the theoretical basis on a simplified version of the system. While making and evaluating prototypes can be time consuming and expensive in the short-term, this approach can be very cost effective in the long term. Working with medical grade, sterilized equipment is expensive. Purchasing such equipment without testing equivalent models can be inefficient and wasteful. Accordingly, a prototype has been designed to assess a specific system configuration that should provide the same consistent backpressure as the water column technique while reducing the amount of space required.

This prototype is intended to backpressure only one bioreactor at this time. A successful test would warrant increasing the number of bioreactors pressurized until system capacity limitations are realized. From any hardware, garden supply, or home improvement store a garden sprayer should be readily available. A garden sprayer consists of a canister typically capable of holding one to two gallons of fluid, a lid with built in pump mechanism, a hose with attached spray trigger and nozzle, and a pressure relief vent. Depending on the design, some of these sprayers have triggers on the pump handle that is attached to the lid – this sprayer design should not be used as it would require taping down the trigger for use without a laboratory assistant. This equipment is typically used for spraying insecticides or fertilizer around the garden or landscape. The pressure vent is a useful feature, but not entirely necessary. Most pressure vents will "pop" or relieve when 45 psi is exceeded within the canister. Some models have this feature and some do not. Regardless, some "x" number of pumps to pressurize the canister can be correlated with a target range for internal pressure using the data acquisition software if need be.

There are a couple of options when it comes to rigging the pressurized canister to the bioreactor. The spray hose can be entirely removed from the sprayer by unscrewing the connecting adaptor. Over the last few years, the hoses used in the system have become much more robust compared to the previous hoses which were not much different than the polymer tubing currently used in the tissue engineering laboratory. The more robust tubing is a little more difficult to work with, but it holds pressure well. If it is too difficult, it can be removed and a new adapter can be fitted to the system. They are typically 3/8 inch to 1/4 inch adaptors. Using tubing with dimensions similar to the silicone tubing of the bioreactor, the adaptor would need to be 3/8 inch to 1/8 inch unless the silicone can stretch to accommodate a slightly larger barb. The other option would require removing the spray wand from the tubing and adapting the original tubing to the next portion of the system.

Either way, the spray canister is then connected to a pressure regulator capable of operating within a 1-5 psi range. A 1-10 psi range would work too if the more precise regulator is not available. Since the goal is to backpressure the system to 100 mmHg, the regulator only needs to pressurize the system to 2 psi. Specific connectors and/or adaptors may need to be used to connect the equipment and tubing. There are specific flanged brass fittings used for gas lines and micro tubing that would need to be used upstream of regulator. This portion of the system would be subject to elevated pressures that the silicone tubing would not withstand (potentially 45 psi). The silicone tubing would balloon and either leak at the fittings or just be blown off altogether. Downstream of the regulator, typical bioreactor materials can be used.

The pressurized canister is then connected to a 3 port reservoir, not unlike the vented reservoir used in previous tests. Once the system is connected, the regulator can be adjusted to provide 2 psi in the system. The regulator would be attached directly to the reservoir because the reservoir acts as the high point of the system and contains a pocket of gas. Once the regulator is opened, there may be an initial redistribution of gases within the system. Running the pump, or priming the pump, should reestablish standard conditions.

Using this technique, the bioreactor setup would not utilize the height of a water column to provide base-line, back pressure levels. The bioreactors and reservoirs would all be maintained on a level surface with backpressure now provided by virtue of pressurized air space in the reservoir.

The volume of air provided by the canister should be more than adequate to pressurize the system for an extended period of time. Tests can be run to quantify the duration of available supply, but even a one gallon canister should maintain pressure while making up losses due to leaks for several days. Also, while the canister can be pressurized to roughly 45 psi, 10psi should easily support a trial. This pressure would be sufficient to easily maintain a 2 psi backpressure. Gases would likely bleed out of the system faster than fluid would (based on previous studies), but tests would have to be run in order to properly characterize the limitations of the setup. It would not be difficult to upgrade the reservoir to improve the seal. Other sources of compressed air are readily available and capable of sustaining the system for longer durations of time. This model is just good for assessing the theory.

Considerations for Future Bioreactor Pressurization

Assuming that the pressurized canister works in pressuring the system, there are a variety of ways to produce a final design. There is a lot of room for customization. There is room to work with system configuration, sources of compressed air, air filtration, interface between air and fluid, and scalability.

The prototype design can easily be scaled up to pressurize multiple systems at once by simply branching the tubing downstream from the regulator off to the other systems. This can be space effective and well organized with a properly designed manifold. A silent lab bench air compressor could be used to provide a continuous source of compressed air and, if desired, can be connected to an air receiver in order to reduce cycling. Pneumatic syringes can be pressurized to deliver media at a rate to maintain 2 psi within the system. If multiple IV bags are used for reservoirs, they can all be placed inside a pressurized canister that would maintain the 2 psi for the system. Filters can be placed in-line downstream of the pressure regulator to provide sterile gas. There are many ways to go about optimizing this system, but using an air compressor, which only takes up 1 cubic foot of space, to pressurize the bioreactors indefinitely would be well worth it.

Backpressure Regulators

The backpressure regulator is a potential solution for establishing a desired base-line pressure and minimizing consumed space. Marc Dawson used a ratchet clamp to restrict flow and back pressure the bioreactor system. The ratchet clamp was able to provide flow resistance and thereby elevate pressures within the systems, but the pressures were hard to accurately set or control. This led to further research of system back-pressuring options – which, in turn, led to research on controlling back pressure through the application of pressure regulating devices. A backpressure regulator is a valve that installs in line on a system subject to pressurization for the express purpose of maintaining a preset pressure upstream (Figure A.3). The back pressure regulator is placed downstream of components that require a nominal pressure higher than the normal unregulated line pressure. The regulator is set to a desired operating level and will then relieve as necessary to maintain desired inlet or upstream pressure. The back pressure regulator is also used to provide high pressure dissipation in order to protect sensitive equipment. If, for whatever reason, target pressure is exceeded, the backpressure regulator will open and release fluid from the system in order to restore the desired maximum pressure level.

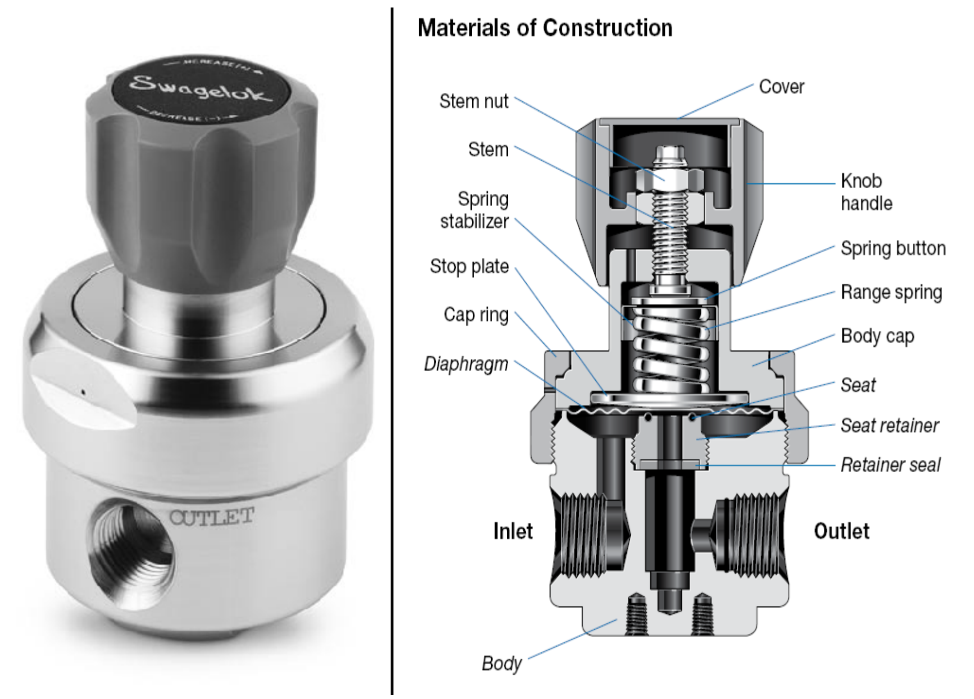

Figure A.3 Back-pressure regulator schematic.

For use in the Cal Poly system, the backpressure regulator would be connected in-line downstream of the bioreactor chamber. The backpressure regulator is set by tightening or loosening its cap. Tightening the cap compresses a spring that seals off flow with a diaphragm. Higher pressures will raise the diaphragm and allow fluid flow. The regulators of interest are able to control pressures through a range of 0 to 10 psi. Roughly 50 mmHg equates to 1 psi. The operational pressure would be at roughly 2 psi in order to maintain a constant pressure of 100 mmHg.

In conversation with technical support from Swagelok, a company who carries a wide variety of backpressure regulators, the practicality of using such devices was reviewed. An estimate for a single regulator was \$200 to \$300 depending on material of construction. The company was unwilling to provide a sample regulator for testing purposes, but was open to the possibility of returning the regulator if it was not what the lab needed (a re-stocking fee would be assessed). The device is autoclavable with a maximum operating temperature of 200 ˚C. Technicians from Swagelok were consulted specifically about the system configuration that was intended to be used. They did not feel that the regulator was sensitive enough to function properly at the range of physiological pressures that the system would be operated at. 2 psi is on the low end of the 0 to 10 psi regulatory ability of the device. In addition, the threading of the regulators determines the degree of control available when setting the pressure limit. Fine tuning on the scale of mmHg may be difficult. Therefore there are a number of potential factors that should be taken into consideration if backpressure regulators are considered for the bioreactor system.

While the backpressure regulator in principal may provide an effective solution, the devices readily available at a reasonable price are not sensitive enough to meet the requirements of lab's application. It would, however, be very interesting to see how well they work when there is more discretionary funding available. The backpressure regulator is still a potential solution for establishing a desired base-line pressure and minimizing consumed space, but the right specifications must be met.

Considerations for Bioreactor Chamber Modifications

The redesigned bioreactor chamber also has room for improvement. The current Lock and Lock bioreactor system has potential to suffer from cell sedimentation during the early stages of vessel cultivation. The circular design of the newly designed bioreactor chamber would allow it to be rotated 360˚. To facilitate this capability, a rack could be designed to suspend the chamber in an individual cradle and allow either manual or automatic rotation. Rotation of 360˚ or any subset thereof could easily be managed. After 360˚ of rotation was achieved, direction of rotation could be reversed and repeated – this way rotary unions are not required and the tubing will not twist excessively. If continual 360˚ rotation is desired, rotary unions would have to be placed at both end of the bioreactor system. There are a number of such fittings on the market. Colder Products Company has a medical grade barbed swivel connector that would be compatible with the bioreactor system. The fitting is referred to as the SRC series (Figure A.4)

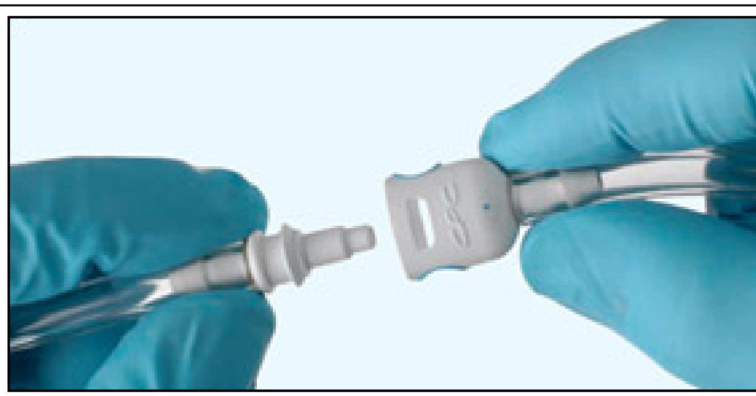

Figure A.4 SRC fitting from Colder Products Company.

In addition, the panel mount luer lock fittings could be replaced. They are not intended to bridge dissimilar environments or maintain any sort of seal. They have worked well enough for laboratory purposes, but bulk head fittings would be better options. They are slightly more expensive, but they are intended to operate under conditions in which two different environments are bridged. The chamber end plates of the newly designed bioreactor chamber have a large enough diameter to accommodate a range of fitting dimensions. The flexible end plates of the bioreactor chamber could be replaced with more rigid materials to accommodate more substantial fittings if necessary. The other upside to the design is that the silicone discs could be made of a thinner, more compliant, and less stiff material. While pulsing pressure waves may cause slight mechanical stimulation, more mechanical stimulation could be applied to the system at later stages of vessel development. The flexible walls would accommodate the attachment of a mechanical apparatus intended to apply cyclic tensile loads to the vessel/scaffold.

Recent findings within the lab have indicated that the endothelial cells have been migrating into the scaffold. Whether this is due to pressure sodding or some other factor, it is unclear. It has been hypothesized that the stretching of the ePTFE scaffold during the loading process may be accountable. The flexible walls of the new bioreactor chamber may potentially alleviate the issue. The walls are flexible, and as such, they do not overly stretch the scaffold.

Considerations for Future Bioreactor Chamber Rack

In an effort to propose future work for this project, initial research and design was performed for the development of a bioreactor chamber rack that would organize the bioreactors in a space saving arrangement, and rotate the chambers to prevent cell sedimentation. These are important parameters as the lab looks to scale up the number of potential tests that could be conducted at a single time. Dr. Kristen Cardinal used to periodically rotate the chambers by hand at set intervals when she was performing her research. This can be time consuming and space limiting depending on stack configuration and the number of chambers. The following design would eliminate the need for manual performance of such operations.

While the most recently designed bioreactor presented in this thesis has square end caps, the end caps could be modified to a circular geometry without much difficulty. The following bioreactor chamber rack design is based on the use of a bioreactor with circular end caps or, at least, circular projections from the end caps. The bioreactor would rest between two slotted walls made of delrin. Delrin, with the proper surface finish, is very slick. It is often used on production line conveyers to facilitate product movement down the line. Once the bioreactor chamber is placed with one end cap in both slots of the opposing walls, a guard is placed over the top of the bioreactor chamber. The guard is slotted just like the supporting walls, but it ensures that the bioreactor does not walk out of the rack as it rotates.

On one side of the rack, a compact DC gearbox motor is attached. These motors are relatively inexpensive, require 12 VDC to operate, and are capable of delivering 20-50 lbs of torque at variable RPM (P/N 6409K13, McMaster-Carr). A carbon-zinc high voltage battery can power the system (P/N 7143K37, McMaster-Carr) – capable of delivering 45 V. These powerful batteries take up minimal space (3.6” x 1.05” x 0.63”). The rotary motor will be linked with all of the bioreactor chambers using a gear and belt system – not unlike a bicycle chain. The bioreactors would have to be equipped with gears on one end, which is easily accomplished. The other option would be to use polyethylene belts. These industrial belts are basically large rubber-bands that are tubular. They coil around the driving motor and then loop over the various components of a system that are desired to turn. The bioreactors would then require a groove or slot to run around the entire circumference of the chamber to act as a guide for the belt. The belt then rotates the bioreactors based on the frictional interface between the rubber and the bioreactor material.

These materials and options are readily available and generally inexpensive. The last issue concerning the rotating bioreactors is the rotation itself. There are two different avenues to take: 180º rotation, or 360º rotation. Rotating the chamber only halfway around and then turning it back to its original position accomplishes all the motion necessary to prevent sedimentation. The problem here, however, is finding and equipping a rotary motor that oscillates between 180º turns and the original set point. The advantage in having a bioreactor that only turns part of the way over and then reverses is that the tubes connecting to it will never tangle and expensive rotary unions are not required. This would justify using such a motor or system, but there is yet a simpler solution out there – swivel connectors. There are a variety of plastic luer lock style fittings that are medical grade and designed to rotate 360º, as mentioned previously. They are generally used for medical equipment to prevent tangling or kinking tubing, but they would work just as well within the bioreactor system. Below is a diagram illustrating the proposed assembly and use of the bioreactor chamber rack (Figure A.5).

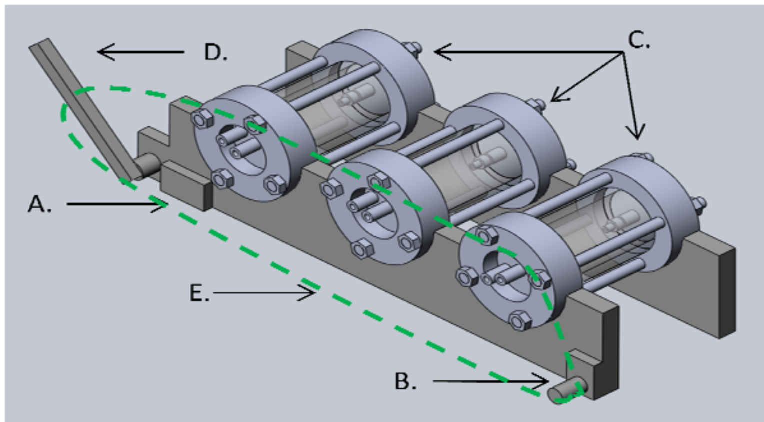

Figure A.5 Bioreactor chamber rotary rack containing: carbon-zinc battery (A), compact DC gear motor (B), bioreactor chambers (C), chain engaging lever (D), and chain path (E).

An additional modification that may be of interest may be to customize the bioreactor chamber rack to allow the bioreactor chambers to be tilted (Figure A.6). Currently, all of the bioreactors lay horizontal and parallel to the flat surface they are placed on. Accordingly, they have orientation in an x and y planes. The addition of a third dimension (z coordinate) would provide unique flow conditions that may better mimic the random geometries found within the human body. This can be done using the rotating bioreactor rack as well. The wall upon which the bioreactors rest would need to be affixed with a binder-style hinge (Figure A.6). Then, one end could be elevated by increasing the supporting wall’s height. The height could be increased by either including adjustable legs or providing a stair-step graduation. As one end elevates, the hinges allow the bioreactor to tilt in accommodation. The slotted grooves and lock-bar would prevent the bioreactor chamber from falling. A basic illustration of this concept is included below.

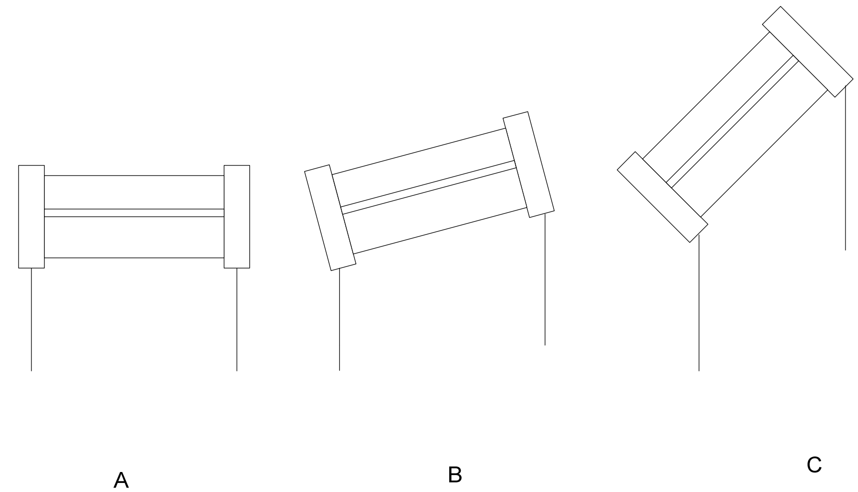

Figure A.6 Three stages of elevating the bioreactor chamber.

Cautionary Note Regarding Bioreactor Stacking

Using the incubator to house a large number of bioreactors is a long-term goal in the BVM laboratory. To accomplish this goal, the setup would have to be highly scalable and consume a minimal amount of space. In the past, this was accomplished this by stacking bioreactor chambers on top of one another. This exposes each chamber to varying pressures because of the transfer of loading forces in the stack. The chamber on bottom of the stack is compressed by the 2 fluid filled chambers on top of it. The solid wall reservoir would not compensate for the compression and the vessel would be exposed to elevated pressures. The height of the stack of bioreactor chambers also has a potentially significant drawback. The top chamber may be in a vacuum if it is above its corresponding reservoir. It would be interesting to see if stack order has influenced results at all.